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THE SUBLETHAL EFFECTS OF DISPERSED OIL ON AN ESTUARINE ISOPOD

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ABSTRACT

The sublethal effects of physically and chemically (Corexit 9527) dispersed Prudhoe Bay crude oil on the physiology and behaviour of the estuarine isopod, Gnorimosphaeroma oregonensis were examined in a minicomputer controlled flow-through system. Test organisms received oil concentrations approximating 0, 3 and 20% of the calculated 48-h LC_{50} for periods of 24 and 48 hours for each dispersion type. The effects of exposure concentration, exposure duration and dispersed oil type on both critical and subcritical sublethal parameters (Percy, 1980) were investigated, as well as the rate and extent of recovery responses.

In general, exposure to both physically and chemically dispersed oil caused a significant increase in oxygen consumption of treated isopods and a concomitant decrease in carbon assimilation rates and efficiencies. In addition, isopods exposed to oil dispersions accumulated naphthalenes in their tissues, and in some cases showed altered moulting patterns and mating responses. The magnitude and persistence of sublethal effects were usually dependent upon oil concentration and exposure duration. In most cases, exposures to chemically dispersed oil resulted in more pronounced sublethal effects than comparable treatments with physically dispersed oil. These sublethal effects, however, usually persisted for less than 48 hours beyond the end of the oil exposure. The significance of the sublethal effects and subsequent recovery responses observed in the test populations are discussed.

RÉSUMÉ

Les effets sublétaux de dispersions par des moyens physiques ou par des moyens chimiques (Corexit 9527) de pétrole brut de la baie Prudhoe sur la physiologie et le comportement d'un isopode estuarien, *Gnorimosphaeroma oregonensis*, ont été étudiés dans un système à écoulement continu régularisé par un mini-ordinateur. Les isopodes cobayes, c'est-à-dire les sujets de l'expérience, ont été exposés à des teneurs en pétrole équivalentes à environ 0,3 et 20 p. cent de la CL_{50} - 48 h pendant des périodes de 24 et 48 heures, pour chaque type de dispersion. On a étudié les effets de la teneur en pétrole, de la durée d'exposition (c.-à-d. d'essai) et du type de dispersion de pétrole sur les paramètres sublétaux critiques et subcritiques (Perey, 1980), de même que la rapidité et l'ampleur des réactions de rétablissement.

D'une façon générale, l'exposition aux dispersions par voie physique ou par voie chimique de pétrole s'est traduite par une augmentation appréciable de la consommation d'oxygène chez les isoposdes testés, accompagnée d'une diminution des taux et efficacités de l'assimilation du carbone. Les isopodes exposés aux dispersions de pétrole ont, de plus, accumulé des naphtalènes dans leurs tissus et présenté, dans certains cas, une altération des processus de mue et d'accouplement. L'importance et la persistance des effets sublétaux étaient généralement fonction de la teneur en pétrole et de la durée d'exposition. L'exposition à la dispersion par voie chimique s'est, dans la majorité des cas, traduite par des effets sublétaux plus marqués que ceux qui furent notés avec la dispersion par voie physique dans des conditions équivalentes. Les effets sublétaux ne persistaient cependant généralement pas plus de 48 heures après la fin de l'exposition. L'ampleur des effets sublétaux et des réactions de rétablissement chez les sujets de l'expérience fait l'objet d'une analyse.

FOREWORD

This report was prepared by W.S. Duval, L.A. Harwood and R.P. Fink of Environmental Sciences Limited, for the Research and Development Division, Environmental Emergency Branch, Environment Canada. Mr. Cal Ross of this branch acted as scientific authority for the project.

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CONCLUSIONS AND RECOMMENDATIONS

In a recent review of the effects of petroleum hydrocarbons and chemical dispersants on benthic and intertidal invertebrates, Percy (1980) emphasized that assessment of the impacts of oil spills must not only consider lethal toxicity, but sublethal effects which may have even greater ecological significance. This author further divided sublethal effects into those which have direct, ecologically significant consequences (critical) and those which only affect individual organisms (subcritical). In the present program, both critical and subcritical effects of physically and chemically dispersed crude oil on an estuarine invertebrate (Gnorimosphaeroma oregonensis) were examined. The program was designed to provide at least partial answers to the following five questions:

1. What concentrations of dispersed oil are required in the water column to produce measurable sublethal effects?
2. Is exposure duration important in determination of the magnitude and persistence of these effects?
3. Do sublethal changes in the physiology and behaviour of isopods persist beyond the period of contact with oil, and if not, what time is required for recovery of affected organisms?
4. Does the use of chemical dispersants cause any different sublethal effects or alter the magnitude and duration of effects observed with physically dispersed oil?
5. In view of the above, what is the potential ecological significance of sublethal changes in the physiology and behaviour of isopods, and will use of chemical dispersants alter these consequences?

Crude oil has been shown to affect a number of biochemical, physiological and behavioural processes in marine invertebrates, although the nature of observed effects have varied with species, life history stage, oil type and exposure conditions. The effects of dispersed oil or its water-soluble constituents on benthic and intertidal crustaceans have included: accumulation of hydrocarbons in tissues by direct absorption from seawater and ingestion with food; changed rates of excretion of dissolved organic and inorganic metabolites; altered respiratory metabolism; induction of neoplastic growth and lesions; altered activity patterns (e.g. swimming, crawling, avoidance); loss of equilibrium; impaired chemoreceptor function; decreased growth; changes in carbon flux or assimilation; altered external pigmentation; decreased or increased moulting; impairment of

mating; and decreased survival of reproductive products (Sanborn and Malins, 1977; Duval and Fink, 1980; Percy, 1980). Not all of these sublethal effects have been observed with isopods, and both Percy and Mullin (1975) and Duval and Fink (1980) provide evidence to suggest that isopods may be considerably more resistant to the impacts of oil than other crustaceans.

Although the type, concentration and duration of oil exposure influenced either the types or magnitude of sublethal effects observed with G. oregonensis in this study; the following physiological and behavioural effects were encompassed:

- increased respiration rates;
- decreased swimming and crawling activity;
- avoidance responses;
- reduced frequency of mating (or pairing);
- loss of co-ordinated motor ability;
- increased and decreased moulting;
- decreased carbon assimilation rates;
- decreased carbon assimilation efficiencies; and
- uptake of naphthalenes.

At the same time, however, the results of this study indicate that a short-term exposure of Gnorimosphaeroma to physically or chemically dispersed Prudhoe Bay crude did not have a significant long-term effect on either growth or reproductive success. Most of the sublethal effects of oil-in-water dispersion (OWD) exposure were previously documented in a study of the effects of the water soluble fraction (WSF) of Prudhoe Bay crude oil on the same species (Duval and Fink, 1980), although trends noted in this earlier study were not as clearly defined. Duval and Fink (1980) also reported that exposure of G. oregonensis to water-soluble hydrocarbons increased excretion of dissolved organic metabolites. They speculated that this process may be involved in depuration of hydrocarbons accumulated in the tissues of test organisms; however, this was not examined in the present investigation.

Both dispersed oil concentration and exposure duration influenced the type, magnitude and persistence of sublethal physiological and behavioural effects. At the lowest concentration of physically dispersed oil examined (2.2 ppm) and when the exposure duration was 24-h, the only significant effect observed was a slight increase (13.2%) in respiration rate. As discussed by Percy (1980), changes in an organism's respiratory

metabolism by itself is considered a subcritical effect, and is of limited ecological consequence unless persisted for sufficient periods to subsequently affect other critical processes such as carbon assimilation or growth. At this low dispersed oil concentration and short (24-h) exposure period, respiratory recovery also occurred within 8 hours of the end of treatment, substantiating the subcritical nature of this type of effect under short-term, low concentration exposure conditions. However, at an equivalent concentration of chemically dispersed oil (1.1 ppm) in terms of a proportion (3%) of the 48-h LC_{50}, there was significant uptake of naphthalenes by treated isopods and no depuration within the subsequent 48-h recovery period. Although respiration rates also increased slightly (4.2%) during this treatment and in the following 24 hours (9.2%), this difference was not statistically significant. The reason for the significant uptake of naphthalenes at a dispersed oil concentration which on a volumetric basis was 50% lower than with the physically dispersed crude, was almost certainly related to the smaller oil droplet size produced when Corexit 9527 was used to disperse the oil. At equivalent concentrations of chemically and physically dispersed Prudhoe Bay crude oil, the chemically prepared dispersion had 7.5 times more oil particles and 2.8 times greater surface area, thereby increasing the opportunity for contact with test organisms and potential for uptake of hydrocarbons by absorption through body surfaces.

At these same concentrations of physically and chemically dispersed oil (2.2 and 1.1 ppm, respectively), increasing the exposure duration from 24-h to 48-h resulted in more pronounced subcritical (i.e. respiration) effects, as well as an additional critical effect in the form of decreased rates of carbon assimilation. The effect of physically dispersed oil on respiration of Gnorimosphaeroma was progressive, and on the second day of exposure was 22% higher than normal. However, as in the case of the 24-h exposures, respiratory recovery was complete within 4 to 8 hours. Although not statistically significant, 48-h exposure to 2.2 ppm of physically dispersed oil also resulted in a relatively pronounced depression in both assimilation rate and efficiency. Both of these sublethal effects would be considered critical according to the criteria discussed by Percy (1980), but each returned to normal during the 48-h post-exposure phase of the study. The greater respiratory expenditure observed during the second 24-h of treatment was the probable cause of the decreased assimilation rate, while the decreased assimilation efficiency may have been related to a decrease in the feeding rate of treated isopods. In contrast to the results observed during the 24-h exposure to physically dispersed crude, there was a significant uptake of naphthalenes during the longer exposure, and similarly

no depuration within 48 hours of the end of treatment. Since metabolism of accumulated hydrocarbons requires energy in excess of the amount necessary for basal metabolism (Lee et al., 1972), the concomitant increase in oxygen consumption observed during each day of exposure (5.7 and 22.3%, respectively) may have been associated with the initial phases of hydrocarbon metabolism and, therefore, resulted in decreased net carbon assimilation.

The types of sublethal effects observed during the 48-h exposure to chemically dispersed Prudhoe Bay crude at a concentration of 1.1 ppm were similar to those found during the shorter exposure, but of greater magnitude. In the 24-h exposure, respiration only increased 4.2% and was not significantly different from control levels, whereas in this study 9.3 and 17.7% increases in oxygen consumption were observed on successive exposure days. Recovery was also slower than observed following the shorter exposure to the same concentration of chemically dispersed oil or the 48-h exposure to physically dispersed oil. The effects of this treatment on assimilation rate, assimilation efficiency and uptake of naphthalenes were almost identical to those observed during the shorter exposure. Both assimilation rate and efficiency decreased during treatment and returned to normal during the recovery phase, while significant naphthalene uptake without depuration was observed.

Increasing the concentration of physically and chemically dispersed oil to 20% of the 48-h LC_{50} resulted in pronounced increases in the sublethal physiological and behavioural effects, particularly in the case of the Corexit 9527/Prudhoe Bay crude oil mixtures. As found at 3% of the acute lethal concentrations of the different dispersion types, increasing exposure duration also had a tendency to accentuate these effects. Both 24-h and 48-h exposures to physically (13.2 ppm) and chemically (6.6 ppm) dispersed Prudhoe Bay crude oil resulted in marked accumulation of naphthalenes in the tissues of Gnorimosphaeroma. Uptake of naphthalenes was also concentration-dependent and significantly greater than that observed at the lower concentrations of both dispersion types. No significant depuration of these hydrocarbons was noted within the 48-h recovery period, although the results of long-term studies which were completed at similar concentrations (27% 48-h LC_{50}) indicated that depuration occurred within 8 days.

Assimilation rates and efficiencies of G. oregonensis were also markedly reduced by exposure to the higher concentrations of physically and chemically dispersed crude oil. However, determination of the statistical significance of assimilation data was limited by the variability in the data, typical of carbon flux studies. Chemically dispersed oil had a tendency to depress assimilation to the greatest extent, particularly during the

48-h exposure to 6.6 ppm where rates were 71% lower than normal. Depression of carbon assimilation by this amount would clearly be considered of critical ecological significance, but as in the case of the experiment conducted at the lower concentration (1.1 ppm), rates returned to normal within 48 hours of treatment. A reduction in assimilation was also noted during the 48-h exposure to 13.2 ppm of physically dispersed oil. In this case there was no evidence of recovery during the post-exposure phase of the experiment; the reason for lack of recovery is unknown.

The effects of the higher oil concentrations on oxygen consumption of Gnorimosphaeroma were both exposure duration- and concentration-dependent, with chemically dispersed oil causing the most pronounced increases in respiratory metabolism. At these concentrations, the effects of chemically dispersed oil on oxygen consumption were apparent within four hours of the beginning of treatment, and on the average, were 25.8% to 30.1% higher than normal during the first exposure day and 55.3% above control levels on the subsequent exposure day. Respiratory recovery was also not as rapid or complete as observed at the lower concentration of chemically dispersed oil, with rates remaining 15.5 to 43.8% higher than in untreated isopods on the first post-exposure day and then decreasing to 14.8 to 23.3% above normal during the remainder of the experiments. Nevertheless, all studies completed with the Corexit 9527/Prudhoe Bay crude oil mixtures did indicate that respiratory recovery was at least in progress near the end of the experiments.

A number of studies have shown that exposure of benthic crustaceans to petroleum hydrocarbons can delay or in some cases completely inhibit moulting (Caldwell et al., 1977; Wells, 1972). Mecklenberg et al. 1977 (cited in Percy, 1980) speculated that the sensitivity of crustaceans may be related to an increased permeability of the exoskeleton during moulting which facilitates penetration of toxic compounds into the tissues. In the chemically dispersed oil bioassay, moulting was immediately and severely reduced at concentrations greater than 28.1 ppm. By comparison, physically dispersed oil at a concentration of 3 ppm stimulated moulting of Gnorimosphaeroma, while higher concentrations (12.5 to 59.3 ppm) reduced moulting, but not to the same degree as equivalent concentrations of chemically dispersed crude oil. Stimulation of moulting by petroleum hydrocarbons has been observed by other authors (e.g. Percy, 1978), and also in a study completed with the same species and the WSF of Prudhoe Bay crude oil (Duval and Fink, 1980). Since growth and the moult cycle of crustaceans are closely interdependent, reductions in moulting frequency to the extent observed in the present investigation would

clearly be considered a critical effect according to the criteria of Percy (1980). However, the long-term studies indicated that a shorter exposure (24-h) to almost the same concentrations almost the same concentrations (27% 48-h LC_{50}) of physically or chemically dispersed oil caused no significant reduction in the growth of G. oregonensis over an eight week period.

Reduced mating has been reported in previous investigations (e.g. Atema et al., 1973), and has been suggested to be related to interference with chemotactic responses. Decreased mating was observed during the bioassays with both physically and chemically dispersed oil, although effects were more pronounced and occurred at lower concentrations with the chemically dispersed oil. Reduced mating is also considered a critical effect (Percy, 1980), but in the long-term studies there was no evidence to suggest that exposure to either dispersion type at a concentration of 27% of the 48-h LC_{50} affected the number of emergent juveniles during 8 weeks of observation.

In summary, measurable sublethal effects were observed at physically and chemically dispersed oil concentrations as low as 3% of the 48-h LC_{50} (2.2 and 1.1 ppm, respectively). As exposure duration and/or concentration were increased, sublethal effects changed from very minor and short-term stimulation of respiratory metabolism (a subcritical effect) to decreased rates of carbon assimilation and efficiency, and uptake of naphthalenes (critical effects). In all cases, the most pronounced and persistent effects were found when Gnorimosphaeroma were exposed to Corexit 9527/Prudhoe Bay crude oil mixtures. The time required for recovery of treated isopods varied with the oil type, concentration, exposure duration, as well as the physiological behavioural function affected. Oxygen consumption and assimilation rates generally returned to normal within 48 hours of the end of oil exposure, while depuration of naphthalenes required between 2 and 8 days. The results of long-term studies which involved 24-h exposures to physically and chemically dispersed oil at concentrations equivalent to 27% of the 48-h LC_{50} and eight weeks of subsequent measurements, did not indicate any significant effects from the oil exposure on growth, frequency of moulting and number of emergent juveniles.

Interpretation of the results of the present investigation is limited by the general lack of data describing the concentrations and persistence of emulsified oil in nearshore environments following oil spills. Factors such as amount and type of oil spilled, geographical features of the area, tidal action, ambient weather, cleanup responses, and time of year can all influence the type and degree of oil contamination. Nevertheless, laboratory studies and simulated spills suggest that concentrations of oil in

the upper portion of the water column following an open-ocean spill may be comparable to the range of concentrations tested during the present investigation. However, simulated spill experiments such as those conducted by Cormack and Nichols (1977) indicate that these oil concentrations in the water column would generally only persist for an hour or less. In an estuarine environment, where the capacity for oil retention could be high and the opportunity for dilution relatively limited, dispersed oil concentrations in the range examined here could conceivably persist for longer periods. The authors speculate that the range of concentrations (1.1-13.2 ppm) and durations (24-48 h) of exposure to dispersed oil investigated during our studies are possible following oil contamination in an estuarine marsh such as the Squamish River estuary.

The results of this investigation indicate that use of chemical dispersants in the cleanup response may lead to more pronounced sublethal effects on some estuarine crustacea than naturally dispersed slicks. This trend was probably related to the greater dissolution of water-soluble constituents of the crude oil and/or contact of the organisms with individual oil particles. The ecological consequences of exposure to either form of dispersed oil, however, would be highly dependent on the concentrations and persistence of oil in shoreline environments. Increases in exposure concentration or duration would both increase the probability of ecologically critical behavioural and physiological effects. Nevertheless, the present study also suggests that at least in the case of Gnorimosphaeroma oregonensis, even the critical sublethal effects caused by short-term exposure to emulsified oil would be of relatively short duration and would not necessarily affect growth and survival.

1 INTRODUCTION

During recent years, there has been a steadily increasing emphasis on measurement of the sublethal effects of acute and chronic oil exposure on marine flora and fauna. Studies of the acute lethal toxicity of various oil and dispersant types are of value in assessing the relative sensitivity of different organisms and the potency of different toxicants. However, information of greater ecological relevance can be obtained from sublethal research programs directed at measurement of physiological and behavioural processes affecting growth, survival, reproductive capacity and productivity (Percy, 1980). Of equal importance in the assessment of impacts of oil spills or use of chemical dispersants in the cleanup response, is the time required for recovery of affected resources. The ecological and bioenergetic implications of oil spills and dispersant use are both highly dependent on the persistence of sublethal effects. Of particular importance in this regard is the potential for increasing both the duration and magnitude of impacts when dispersants are used in the cleanup response.

The present research program was designed to assess the nature, magnitude and duration of sublethal physiological and behavioural effects resulting from relatively short-term exposure of an intertidal invertebrate to physically and chemically prepared oil-in-water dispersions (OWD) of Prudhoe Bay crude oil. An intertidal invertebrate was selected as the test organism because of the documented susceptibility of intertidal/estuarine habitats to oil spills (Clark and Finley, 1977; Sanborn, 1977), and the potential persistance of emulsified oil in such habitats following spills and dispersant application. The estuarine isopod, Gnorimosphaeroma oregonensis, was used in study, partly because of its abundance in the study area, and in order to facilitate comparison with the results of a previous investigation by the authors with this species and the water-soluble fraction (WSF) of Prudhoe Bay crude oil (Duval and Fink, 1980). Since the results of this and other studies completed with benthic and intertidal invertebrates (Percy and Mullin, 1975; 1977) have suggested that isopods are relatively resistant to oil. Another objective of the present program was to examine if use of chemical dispersants altered the sensitivity of isopods to oil. Prudhoe Bay crude oil and Corexit 9527 were selected for the investigation because there is an existing literature (e.g. Foy, 1978; 1979) on the toxic and sublethal effects of this crude oil and dispersant to allow comparison with the present results.

The experimental design and apparatus used in this study were similar to those employed in the previous sublethal research program completed by the authors (Duval and

Fink, 1980; Duval et al., 1980). However, in the present investigation, greater emphasis was placed on measurement of both "subcritical" (e.g. respiration, activity) and "critical" (e.g. growth, carbon assimilation, moulting, reproductive success, mating) sublethal effects (Percy, 1980). The study was conducted as part of the Arctic Marine Oilspill Program during a ten week period at Simon Fraser University, Burnaby, British Columbia.

2 METHODS

2.1 Basic Experimental Design

The sublethal effects of physically and chemically (Corexit 9527) dispersed Prudhoe Bay crude oil on the physiology and behaviour of the estuarine isopod, Gnorimosphaeroma oregonensis, were studied in this program. During eight separate five to six day experiments, test organisms were exposed to concentrations of 3 and 20% of the calculated 48-h LC_{50} of each dispersed oil type for periods of 24 and 48 hours (Table 1). The acute lethal concentrations of physically and chemically dispersed Prudhoe Bay crude oil to G. oregonensis were calculated at 48 and 96 hours following completion of flow-through bioassays (see Section 2.5). An additional bioassay was conducted to determine the acute lethal concentration of Corexit 9527 (without crude oil) to the test organisms. A dispersant:crude oil ratio of 1:10 was maintained during all studies involving chemically dispersed oil. Parameters measured during the sublethal studies included respiration rate, carbon assimilation rate and efficiency, and the uptake and depuration of naphthalenes. In addition, observations of mating activity, moulting, mortality and the general activity level of test organisms were recorded on a daily basis. These eight short-term studies were completed in a mini-computer controlled flow-through system previously described by Duval and Fink (1980) and briefly discussed in subsequent sections.

TABLE 1 EXPERIMENTAL DESIGN OF SUBLETHAL STUDIES WITH Gnorimosphaeroma oregonensis (ISOPODA)

Oil Dispersion Type	Exposure Duration (h)	Exposure Concentrations (ppm)
Physical	24	2.2 (3% 48-h LC_{50})
	48	2.2
	24	13.2 (20% 48-h LC_{50})
	48	13.2
Chemical	24	1.1 (3% 48-h LC_{50})
	48	1.1
	24	6.6 (20% 48-h LC_{50})
	48	6.6

Each experiment included a 20 hour acclimation period prior to the initiation of the pre-exposure phase measurements and feeding schedules. Although the experimental apparatus consisted of eight 8-L exposure vessels, respiration rates were only determined in four of these vessels due to limitations in software. Respiration rates were measured at 30 minute intervals throughout each experiment, which consisted of a 48 hour pre-exposure period, a 24 or 48 hour treatment phase, and a 48 hour post-exposure period to measure recovery responses of the test organisms. Isopods in the four vessels not used for respiration rate determinations also received a 48 hour pre-exposure period before addition of the physically or chemically dispersed oil. However, test organisms were removed from this second set of vessels at the end of oil treatment to allow comparison of carbon assimilation rates and concentrations of naphthalenes in tissues before and after the 48 hour recovery period.

In addition to these short-term sublethal studies, the long-term (eight weeks) effects of short-term (24-h) exposure of Gnorimosphaeroma to either physically or chemically dispersed crude oil were also examined. Parameters measured each week following the initial treatment included residual naphthalenes in tissue, length and weight of a subsample of isopods, mortality (including differences by sex), and the number of juveniles present in test populations.

2.2 Collection and Holding of Test Organisms

Gnorimosphaeroma were collected from the tidal marsh of the east delta of the Squamish River estuary between April and June 1980. Collected isopods were identified as Gnorimosphaeroma oregonensis (Dana) using Schultz (1969) and Menzies (1954). Intertidal collection areas were characterized by abundant pools and tidal channels containing submerged stumps, log debris, and sulphide-rich sediments. Water chemistry analyses (Hach DREL/4) completed in the area did not indicate the presence of any contaminants which may have stressed test organisms prior to use in the sublethal studies (see Table 2).

Isopods were collected at low tide from the undersurfaces of submerged log and bark debris, transported to the Simon Fraser University laboratory in 10-L plastic pails, and held for 18-24 hours prior to the initiation of each experiment. During this holding period, the water containing the test organisms was continuously aerated and kept at room temperature (16-18°C). Approximately 100 g of isopods were collected for each experiment, and any observed changes in the sex ratio (or presence of juveniles in the natural population) were recorded during the course of the 3-month study.

TABLE 2 WATER CHEMISTRY ANALYSES OF SQUAMISH RIVER ESTUARY COLLECTION AREA, EAST DELTA/LOW TIDE 27-06-80

Parameter	Level
H_2S (undisturbed)	n.d.
H_2S (sediment disturbance)	1.0 mg/L
Bromine	0.14 mg/L
Chlorine	0.04 mg/L
Chromium (hexavalent)	0.001 mg/L
Colour	40 APHA
Conductivity	4900 µmhos/cm
Copper	0.05 mg/L
Iodine	<0.2 mg/L
Iron (total)	0.12 mg/L
Manganese	0.3 mg/L
Nitrogen (Ammonia)	0.64 mg/L
Nitrogen (Nitrate)	1.9 mg/L
Nitrogen (Nitrite)	0.06 mg/L
Phosphorus, reactive	0.72 mg/L
Silica	2.35 mg/L
Sulphate	155 mg/L
Turbidity	12 FTU

Following the holding period, Gnorimosphaeroma were acclimated to the experimental temperature of 11-14°C over an additional period of approximately 20 hours. This acclimation phase was completed in the exposure vessels described in Section 2.4.

2.3 Preparation of Oil-in-Water Dispersions

A 45-L supply of Prudhoe Bay (Alaskan North Slope) crude oil was provided by the Atlantic Richfield Refinery, Cherry Point, Washington. The oil was transferred from 5 gallon stainless steel shipping containers to 1- or 2-L glass Erlenmeyer flasks prior to initiation of the research program. Each flask was stoppered and covered with aluminum foil to prevent evaporation and photooxidation of volatile hydrocarbons.

A Manostat 2-channel cassette pump was used during preparation of physical oil dispersions to hydraulically displace the oil from the 1- or 2-L flasks into a 15-L insulated glass mixing chamber. Depending on the OWD concentration required, one or two channels of the peristaltic pump were used to displace the oil, and flow rates were varied from 0.5 to 1.0 mL/min to produce oil concentrations from 500 to 1 000 ppm in the mixing chamber. The incoming oil was continually dispersed in fresh seawater with a Caframo Type RZR1-64 stirrer operated at 1 500 rpm. As the volume of dispersed oil decreased following use during each exposure cycle of the experiments, a float-activated switch initiated the addition of more seawater and oil to the mixing chamber. Seawater from a 200-L header entered this chamber by gravity flow through the use of a solenoid-controlled valve connected to a microswitch level control (see Figure 1). The range of toxicant concentrations necessary for the different experiments was achieved through adjustment of the cassette pump speed, and by varying the ratio of toxicant:seawater metered to each exposure vessel.

The chemical oil dispersant (Corexit 9527) was provided by the Canadian Coast Guard and Exxon Corporation, Chemicals Division. Chemically dispersed oil was prepared in a manner similar to physically dispersed oil with the following exceptions. A 10-channel Manostat cassette pump was used to hydraulically displace the oil (2 channels) and directly pump the Corexit (1-channel) to the 15-L glass mixing chamber. Use of different sizes of tubing produced the desired ratio of 10 parts oil to 1 part dispersant. Oil and dispersant flows were combined in a common line, allowing contact for at least 2 minutes prior to being added to the mixing chamber.

During the exposure phase of the studies, a predetermined volume of dispersed oil diluted with seawater was added to the 8-L exposure vessels every 30 min. As a result, oil concentrations gradually increased with time and reached an equilibrium approximately 10 hours after toxicant addition began. Oil concentration in the exposure vessels also gradually decreased with time at the end of the exposure phase.

2.4 Experimental Apparatus

The sublethal effects of physically and chemically dispersed Prudhoe Bay crude oil on respiration rates of the estuarine isopod, G. oregonensis, were measured in four 8-L flow-through vessels. An additional set of four 8-L vessels were used to examine carbon assimilation and residual napthalenes in tissues of test organisms up to the end of the exposure period.

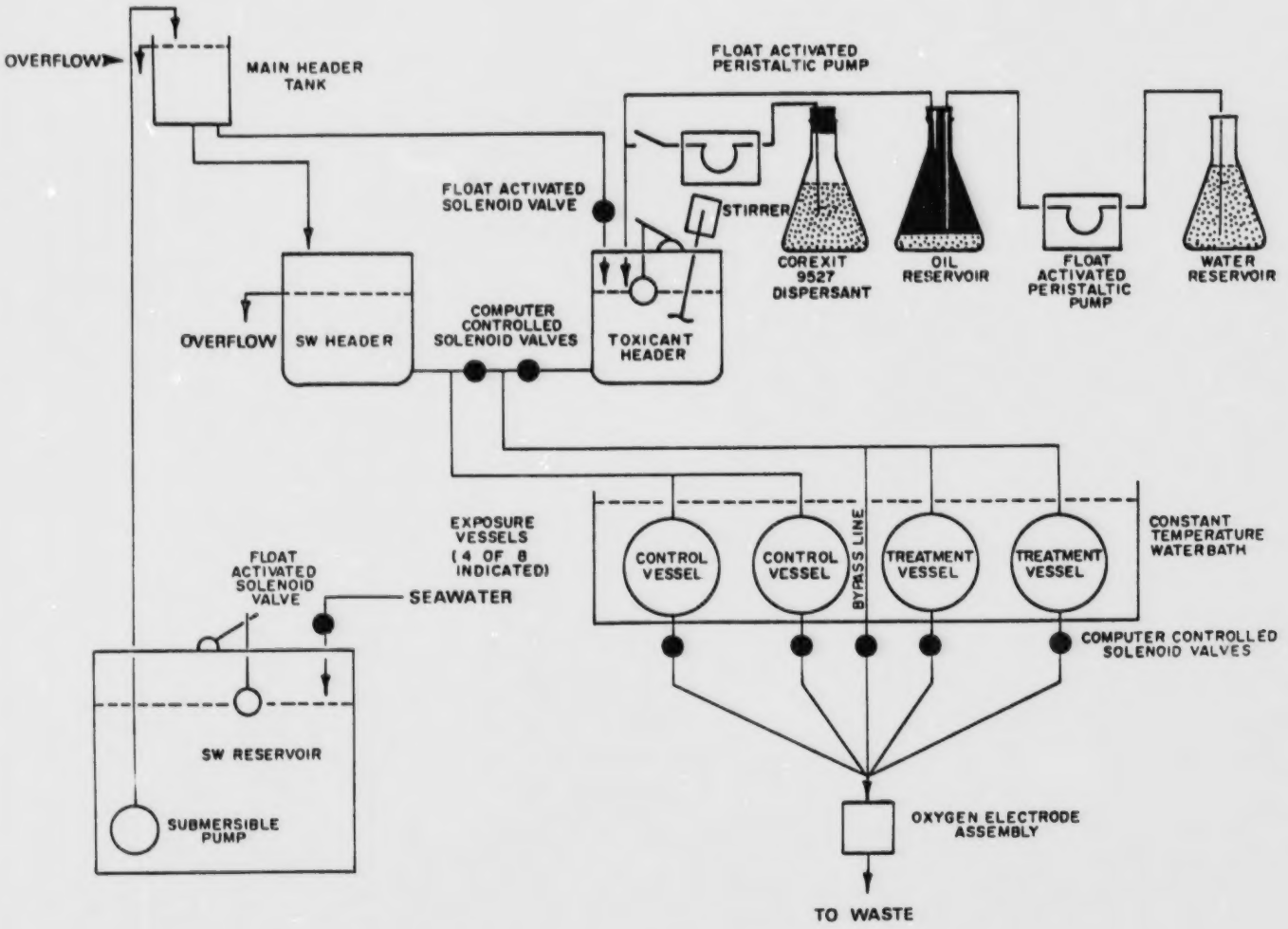

FIGURE 1 SCHEMATIC OF "OWD" PREPARATION AND DOSING SYSTEM

The experimental apparatus used for this research program was similar to that described by Duval and Fink (1980) in a study of the sublethal effects of the water soluble fraction of Prudhoe Bay crude oil on various marine fauna. The following section briefly outlines the major components of this system as well as modifications required for the present research program. Specific details regarding the construction and operation of the respirometer and associated apparatus are available in Duval and Fink (1980) or Duval et al. (1980).

A 200-L seawater reservoir supplied both the toxicant preparation system and a recirculating seawater header located above the respirometer water bath (Figure 1). The gravity-induced flow of seawater and dispersed oil from the headers was regulated through a series of stainless steel solenoid valves operated under computer control. The concentration of dispersed oil entering each exposure vessel was determined by the time (s) that each valve in the apparatus was activated, and was monitored on a periodic basis with a Model TA II Coulter Counter. Four 8-L respirometers (2 control, 2 treatment) were mounted in a temperature-regulated water bath below the seawater and toxicant headers. Seawater and physically or chemically dispersed oil were purged through each vessel to an oxygen electrode assembly where dissolved oxygen concentration was measured. The difference in oxygen concentration between water purged from the vessels and that of air-saturated seawater in the headers, was used to calculate respiration rates of isopods in $\mu L\ O_2/g$ wet weight/min.

A 24-h timer was used to control the photoperiod (12L:12D) of the overhead fluorescent lights in the laboratory. The period of daylight extended from 0800 to 2000 hours, with light intensities at the respirometers ranging from 11 to 15 microeinsteins/m^2/min. Temperatures in the respirometer water bath and seawater header varied from 11-14°C throughout the course of the investigation.

Data acquisition components of the apparatus were centered around a Data General Nova 2/10 mini-computer with 16K words of core memory. A Data General Model 6030 Dual Diskette subassembly was used to load the main control program and various system diagnostics, while a Model 6040 Dasher 60 cps terminal printer with keyboard was used for communications with the mini-computer and recording of experimental results. The major control program which regulated the progress of the experiments and acquired data at specified time intervals (30 min) was written in Assembler under a Real Time Operating System (RTOS). A YSI Model 53 Oxygen Monitor was modified to accept four Model 4304 polarographic electrodes, and the probes placed

in perspex venturies to minimize the effects of residual oxygen consumption. The mini-computer was also used to monitor and compensate for calibration drift in each of the probes as well as to perform an internal self-check feature of the YSI Oxygen Monitor called an A-B test.

2.5 Acute Lethal Bioassays

Flow-through bioassays (96-h) were conducted prior to initiation of the short- and long-term sublethal studies to determine the acute lethal concentration of physically and chemically dispersed oil to the estuarine isopod, G. oregonensis. Three and 20% of the 48-h LC_{50} values for each dispersed oil type were selected as the exposure concentrations for the sublethal studies. An additional flow-through bioassay was conducted to estimate the acute lethal concentration of Corexit 9527 to the test organisms.

All bioassays were conducted in the eight 8-L exposure vessels. Isopods in six of the vessels were exposed to a range of OWD concentrations, while two vessels were used as controls and did not receive toxicant. The addition of seawater and seawater containing dispersed oil was controlled by the mini-computer and hardware/software components described earlier. Concentrations of dispersed oil in the exposure vessels periodically was measured on a volumetric basis with a Model TA II Coulter Counter. This technique was selected in favour of alternative chemical analyses, since it provides a more accurate measure of total particulate oil concentration, and at the same time facilitates examination of particle size distributions in the emulsified oil.

Approximately 100 g of isopods were placed in each exposure vessel, and mortality recorded on a logarithmic time scale according to the procedure of Sprague (1969). General activity, mating behaviour, moulting and external colouration were also recorded during each observation period. After 96 hours, isopods were removed from the exposure vessels and percent mortality calculated for each OWD concentration and observation period. The lethal concentrations (48-h and 96-h LC_{50}) of physically and chemically dispersed Prudhoe Bay crude oil to Gnorimosphaeroma were determined by the graphical method of Litchfield and Wilcoxon (1949).

2.6 Long-term Sublethal Study

In addition to studies of the short-term physiological and behavioural effects of dispersed oil, this program included an investigation of the long-term effects of short-term exposure of isopods to sublethal concentrations of physically and chemically

dispersed oil. Parameters measured during an eight week period following a 24-h exposure to each dispersed oil type included concentrations of naphthalenes in the tissues of test organisms, mortality, growth and the estimated number of juveniles.

Six 8-L flow-through vessels, each containing 13 g (wet weight) of pre-acclimated G. oregonensis were used for the exposure phase of the long-term study. Two vessels were left as controls, while pairs of vessels received 18 ppm and 9 ppm (27% 48-h LC_{50}) of physically and chemically dispersed oil, respectively. Immediately following the OWD exposure, isopods from paired vessels (same treatment) were combined, transferred to three 25-L polyethylene tanks and monitored on a weekly basis for eight weeks. These tanks were maintained at a temperature of 16-20°C, continuously aerated, and seawater exchanged six times a day by a flow-through seawater supply. Test organisms in each tank were fed a diet of goldfish and crab tissue; food was added on an "as required" basis and totalled 31 g/tank over the eight week study. Uneaten food was removed each week and replaced with fresh material.

A random sample of 40-50 isopods was removed from each tank once a week. After sorting of the isopods by sex according to anatomical and size characteristics described by Menzies (1954), the total body length of 10 males and 10 females were measured with calipers. The wet and dry weight and residual naphthalene concentration in tissue were measured on the remainder of the weekly sample. Tissue samples were prepared and analyzed for naphthalene content following methods described in Section 2.9. The number and sex of all dead isopods in each tank was recorded prior to their removal every week. The number of juveniles and moult casts in each tank were also estimated during each weekly observation period.

2.7 Measurement of Respiration Rates

The respiration rate ($\mu L\ O_2/g$ wet wt/min) of isopods in the four respirometers was calculated by the mini-computer at 30 minute intervals for the duration of the pre-exposure, exposure and recovery phases of the short-term sublethal studies. This calculation involved comparison of dissolved oxygen concentration in the seawater entering and leaving the respirometers, following corrections for calibration drift. Differences between the dissolved oxygen content of seawater and seawater containing dispersed oil were also considered during calculation of respiration rates (Duval and Fink, 1980).

2.8 Measurement of Assimilation

The assimilation rate and assimilation efficiency of isopods were estimated immediately following exposure to sublethal concentrations of physically and chemically dispersed Prudhoe Bay crude oil, as well as 48 hours after this treatment. Isopods were fed daily rations of ^{14}C-labelled brine shrimp (Artemia salina), and after completion of each experiment, a subsample of test organisms from each vessel was frozen for subsequent radiocarbon analysis.

During the course of the investigation, three separate cultures of ^{14}C-labelled brine shrimp were prepared. In each case, the brine shrimp were allowed to graze on a log phase culture of Dunaliella tertiolecta which had been inoculated with ^{14}C-bicarbonate. The shrimp were removed from the algal cultures after 24 hours and frozen in portions of 20; one portion was then introduced into each vessel during each day of the short-term studies. In addition, at least six samples of 5 brine shrimp were also retained from each batch for subsequent measurement of activity present in the daily ration. The mean activity of brine shrimp in each of three batches was 191 000, 21 300, and 47 000 dose per minute (dpm)/shrimp.

Isopod samples for radiocarbon analysis consisted of 3 males and 3 females dried to a constant weight, weighed on a Mettler H20T precision balance, and oxidized in a Packard Model B0306 Tri-carb sample oxidizer. Oxisorb-CO_2 and Oxiprep-2 were used as the ^{14}C trapping agent and fluorescing agent, respectively. Radioactivity of the combusted samples was determined with a Beckman L3-8000 Scintillation Counter, and resulting activity in counts per minute (cpm) converted to dpm with a Nubec computer program. Assimilation efficiency (% dpm recovered/dpm fed) and assimilation rate (µg C/mg dry wt/day) were then calculated for isopods in each exposure vessel in all experiments.

2.9 Measurement of Tissue Hydrocarbon Concentrations

Residual naphthalenes were measured in isopod tissue frozen immediately following and 48 hours after exposure to sublethal concentrations of physically and chemically dispersed Prudhoe Bay crude oil. In addition, weekly samples of isopods from each long-term study tank were retained for hydrocarbon analysis.

Isopod samples were dry-ice cooled, pulverized with a mortar and pestle, and weighed on a Mettler H2OT precision balance. Sample weights ranged from 0.5 to 1.2 grams. Each sample was transferred to a 20-mL acid-washed vial containing 15 mL of UV

spectrophotometric grade hexanes. The vials were then sealed with aluminum foil, gently shaken for 1 minute, and stored at 4°C for 3 hours.

Lipid and cellular materials were removed from the extracts by passing each sample through a heat-activated alumina column. The eluent was collected in acid-washed vials and volumes standardized to 20 mL with hexane. The percent transmittance of each sample was measured against a hexane blank at 221 nm using a Perkin-Elmer UV Spectrophotometer. The transmittance of each sample was then converted to naphthalene concentration with a calibration curve derived with standards containing from 0.01 to 10 ppm naphthalene in spectrophotometric grade hexane.

2.10 Observation of Test Organisms

General observations of the test organisms were recorded at the same time on each day of the short-term studies. These observations included mortality, number of moults, relative feeding activity, presence of mated pairs, relative swimming activity, colour and general activity level.

3 RESULTS AND DISCUSSION

3.1 Acute Lethal Bioassays

The acute lethal concentrations of physically and chemically (Corexit 9527) dispersed Prudhoe Bay crude oil and the dispersant itself were determined with 96-h flow-through bioassays. Percent isopod mortality at each observation period for each OWD concentration is provided in Table A of the Appendix. Mortality in control vessels never exceeded 3%. The Corexit 9527 alone resulted in no mortality in 96 hours at the highest concentration (1 000 ppm) used in that particular bioassay. Therefore, mortality observed during exposure of the isopods to chemically dispersed oil was not attributed to the presence of the dispersant. The 96-h LC_{50} values and 95% confidence limits for the oil dispersions were estimated by graphical methods (Litchfield and Wilcoxon, 1949), and were 21.5 (7.1-68.0) ppm and 8.2 (3.5-19.4) ppm for the physically and chemically dispersed oil, respectively. Forty-eight hour LC_{50} values were 70 (24-203) ppm and 32 (13-78) ppm for the physical and chemical dispersions, respectively. However, following analytical methods outlined by Litchfield and Wilcoxon (1949), neither the 48-h or 96-h LC_{50} values for physically and chemically dispersed oil were significantly different from each other ($p>0.05$). Additional bioassays would be required to substantiate the apparent higher toxicity of chemically produced oil-in-water dispersions to this species.

Although there is considerable information on the acute toxicity of oil-in-water dispersions to marine organisms, comparison of studies is often complicated by the lack of standard methods (e.g. static vs. flow-through bioassays) and the fact that many authors have not actually monitored the hydrocarbon concentrations in the water column to which the test organisms are exposed. In addition, considerable variability in the composition of oils tested and the use of different test species at various life stages further hampers the interpretation of past study results.

Craddock (1977) summarized the results of numerous laboratory studies on the acute toxicity of various crude oils and refined petroleum products to marine invertebrates. However, the acute lethal effect of dispersed oil on isopods has not been examined in a flow-through system such as that used in the present investigation. Percy and Mullin (1975) reported no mortality after 96 hours in three species of arctic isopods (Mesidotea entomon, M. sibirica, M. sabini) exposed to heavy dispersions (>300 ppm initial dose) of Norman Wells crude oil in static bioassays. In a similar study using a static system, Foy (1978) examined the acute toxicity of Prudhoe Bay crude oil dispersed

mechanically and with Corexit 9527 to three species of arctic amphipods (Onisimus litoralis, Boeckosimus edwardsi, and Anonyx nugax). The 96-h LC_{50} values for oil/water dispersions were in the range from 32 to 55 ppm for all species, and varied from 24-213 ppm for Onisimus and 64-213 ppm for Boeckosimus and Anonyx in oil/Corexit/water dispersions. He suggested a greater proportion of non-toxic hydrocarbons were dispersed in the water column when Corexit was used, thereby lowering the lethal concentrations in comparison to mechanically dispersed oil. The results of both of these studies are inconsistent with the present findings which suggest that Prudhoe Bay crude oil dispersed with Corexit 9527 is slightly more toxic than the physically dispersed oil. This may be due to the differences in toxic load associated with the static and flow-through bioassay techniques. Since the flow-through bioassay technique facilitates continuous renewal of unweathered oil, the exposure-concentration product and effective dose are greater throughout the experiments. Although other factors may have contributed to the higher acute toxicities of dispersed oil observed in the present investigation, it is generally agreed that flow-through bioassays produce a more absolute estimate of toxicity (e.g. Vaughan, 1973).

The present authors do not agree with Foy's (1978) suggestion that use of chemical dispersants will result in a greater proportion of non-toxic hydrocarbons in the water column. Given equal concentrations of oil added to water, use of Corexit 9527 not only increases the total amount of oil present in a dispersed form, but also increases the surface area:volume ratio (by decreasing mean particle volume), affording greater opportunity for dissolution of partially soluble aromatics, which are the primary hydrocarbons responsible for acute toxic effects. Foy (1978) states that even after 3.5 h without agitation, emulsions of Prudhoe Bay crude oil produced with Corexit resulted in as much as 7.5 times more measurable (fluorescence spectroscopy) hydrocarbons in the water column than an equal amount of mechanically dispersed oil. The results of our study also indicated that the mean particle size of chemically dispersed oil was, on the average, 75% smaller than mechanically-produced oil-in-water emulsions. As shown in Figure 2, mean particle volumes of chemically and physically dispersed Prudhoe Bay crude oil were 536 μ^3 ($\emptyset$ = 5.04 μ) and 2154 μ^3 ($\emptyset$ = 8.00 μ), respectively. Consequently, not only is the opportunity for contact with test organisms increased with chemically dispersed oil, but also the effective surface area for dissolution to produce a water-accommodated fraction.

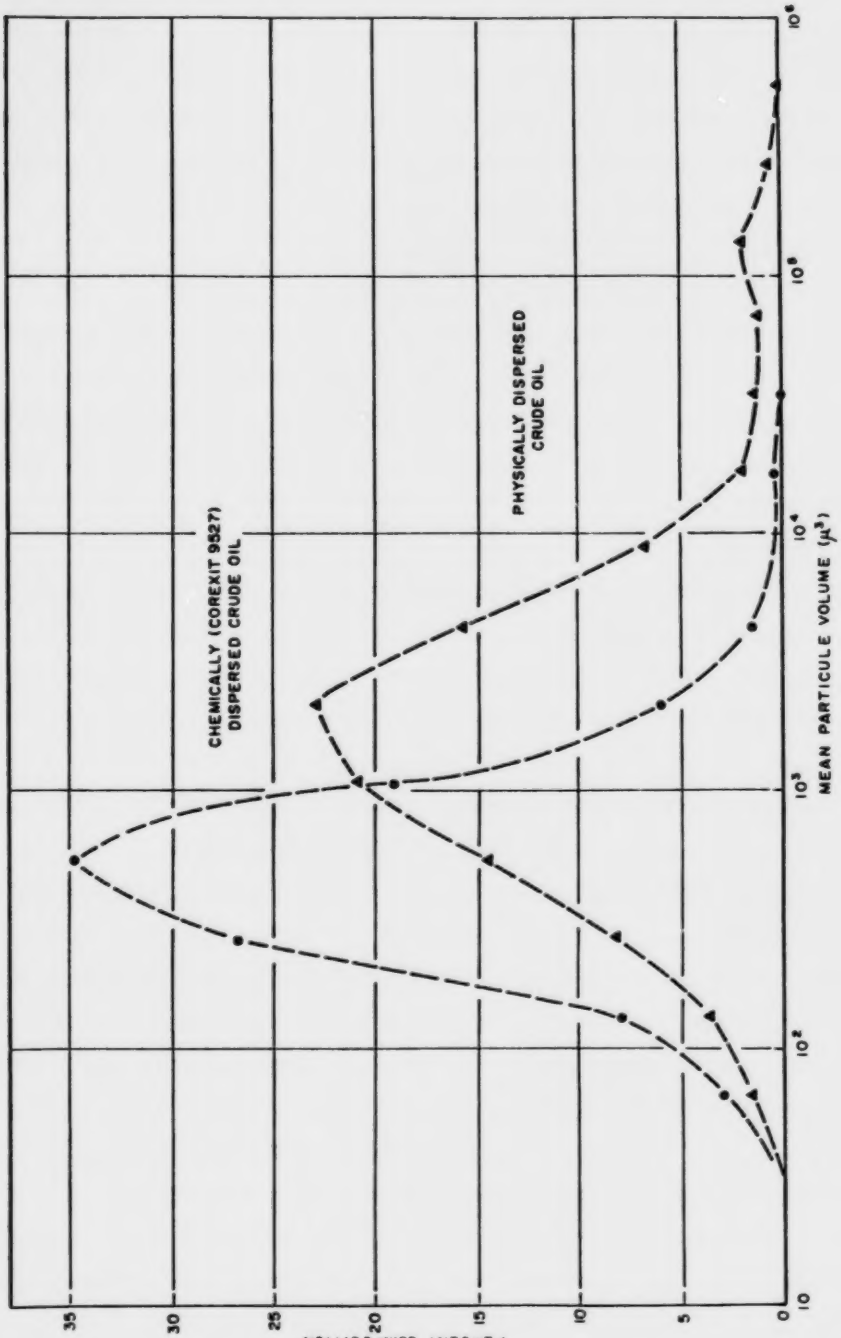

FIGURE 2 PARTICLE SIZE DISTRIBUTIONS OF PHYSICALLY AND CHEMICALLY DISPERSED PRUDHOE BAY CRUDE OIL

The generally more toxic nature of chemically dispersed oil to invertebrates other than isopods has been suggested by other investigators, although differences in oil type and species tested again make comparisons difficult. In a flow-through system, Swedmark et al. (1973) found that oils emulsified with various dispersants were, in general, more toxic to bivalves and crustaceans than crude oil or dispersants alone. In particular, emulsions of Oman crude oil prepared with Corexit 7664 were more toxic to the most sensitive crustacean tested, Leander adspersus (prawn) (96-h LC_{50} = 170 ppm) than mechanically dispersed Oman crude (initial concentration >1 000 ppm).

Sprague (1969) noted that both toxicity curves (median lethal time vs. concentration) and the relationship between percent mortality at various toxicant concentrations and the time of observation can provide insight into the mode of toxic action of various pollutants. The data provided in Figures 3 and 4 suggest that the toxic effects of physically and chemically dispersed oil were generally linear (on a log-probit scale) and had a common mode of action. This conclusion appears warranted because the slopes of these lines did not vary markedly with oil concentration or between the two dispersion types, although the chemically dispersed oil was clearly more toxic at lower concentrations. Toxicity curves with chemically and physically dispersed oil substantiate the latter trend (see Figure 5). At oil concentrations less than 40 ppm, the median survival times were significantly ($p<0.05$) lower when Corexit 9527 was used to disperse the crude oil.

Records of the sex of dead organisms were maintained during the chemically dispersed oil bioassay, and it was found that male isopods suffered considerably higher mortality than females. Similar trends were noted with physically dispersed oil, although the sex of dead organisms was not actually recorded. As shown in Table 3, the lowest two oil concentrations included in the bioassay (5.2 and 12.5 ppm) resulted in only slight mortality to female isopods, but almost complete loss of males. This selective effect on males diminished with increased oil concentration, and chemically dispersed Prudhoe Bay crude at concentrations from 51.6 to 101 ppm was lethal to all individuals of both sexes within 96 hours. The activity of males in the flow-through exposure chambers was also decreased much earlier and at lower concentrations than the females. The greater susceptibility of male Gnorimosphaeroma to dispersed oil was probably related to the timing of the study which coincided with the post-breeding phase of their life history when natural mortality of males was already high and their abundance in the collection area was continuously decreasing.

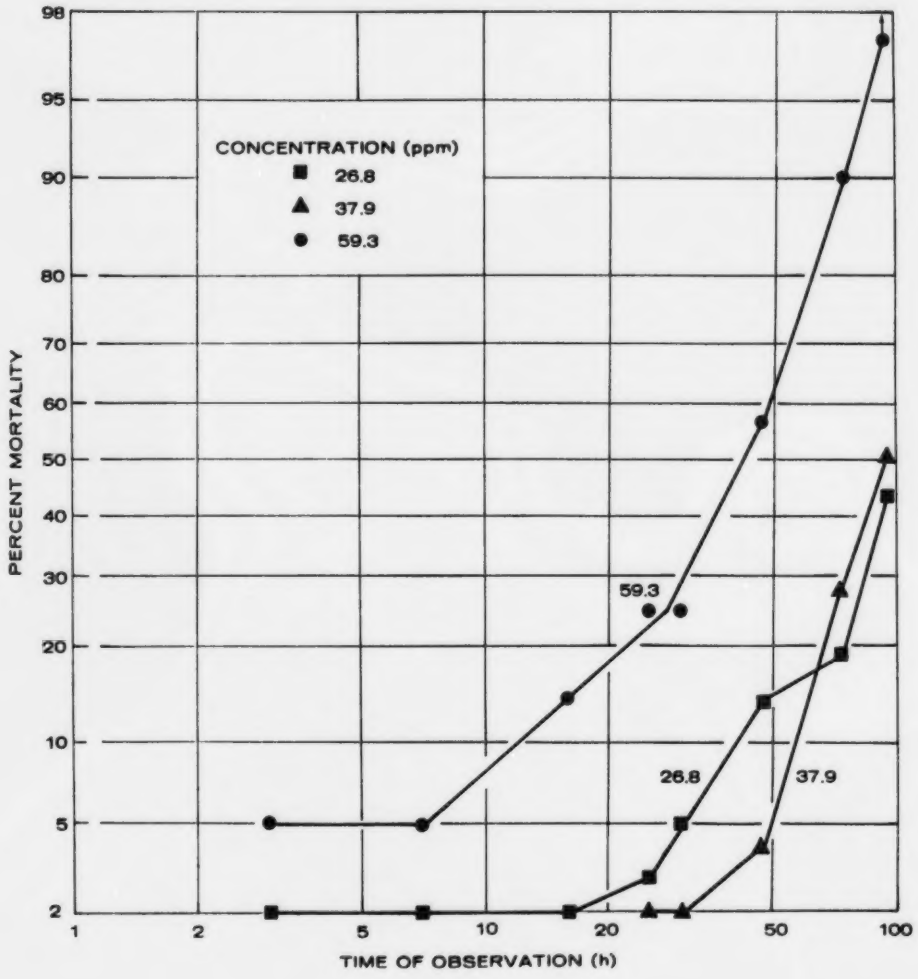

FIGURE 3 TEMPORAL DIFFERENCES IN THE MORTALITY OF Gnorimosphaeroma oregonensis EXPOSED TO VARIOUS CONCENTRATIONS OF PHYSICALLY DISPERSED PRUDHOE BAY CRUDE OIL

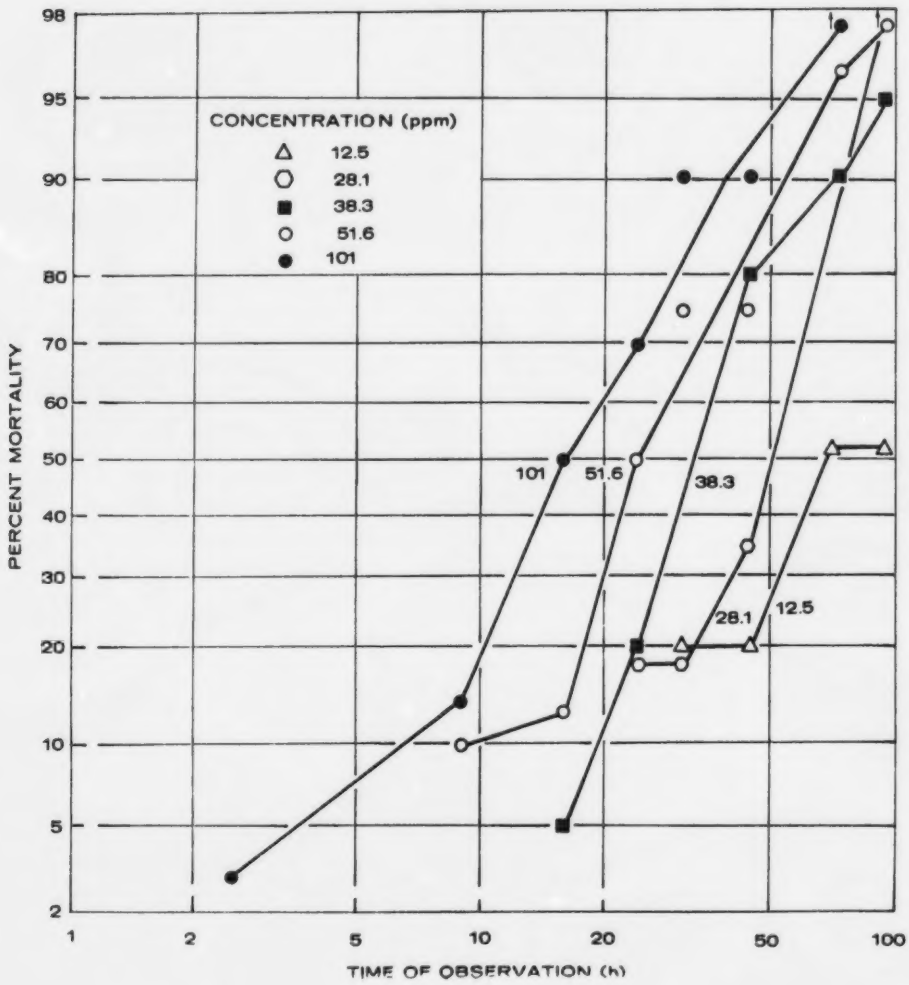

FIGURE 4 TEMPORAL DIFFERENCES IN THE MORTALITY OF Gnorimosphaeroma oregonensis EXPOSED TO VARIOUS CONCENTRATIONS OF CHEMICALLY DISPERSED PRUDHOE BAY CRUDE OIL

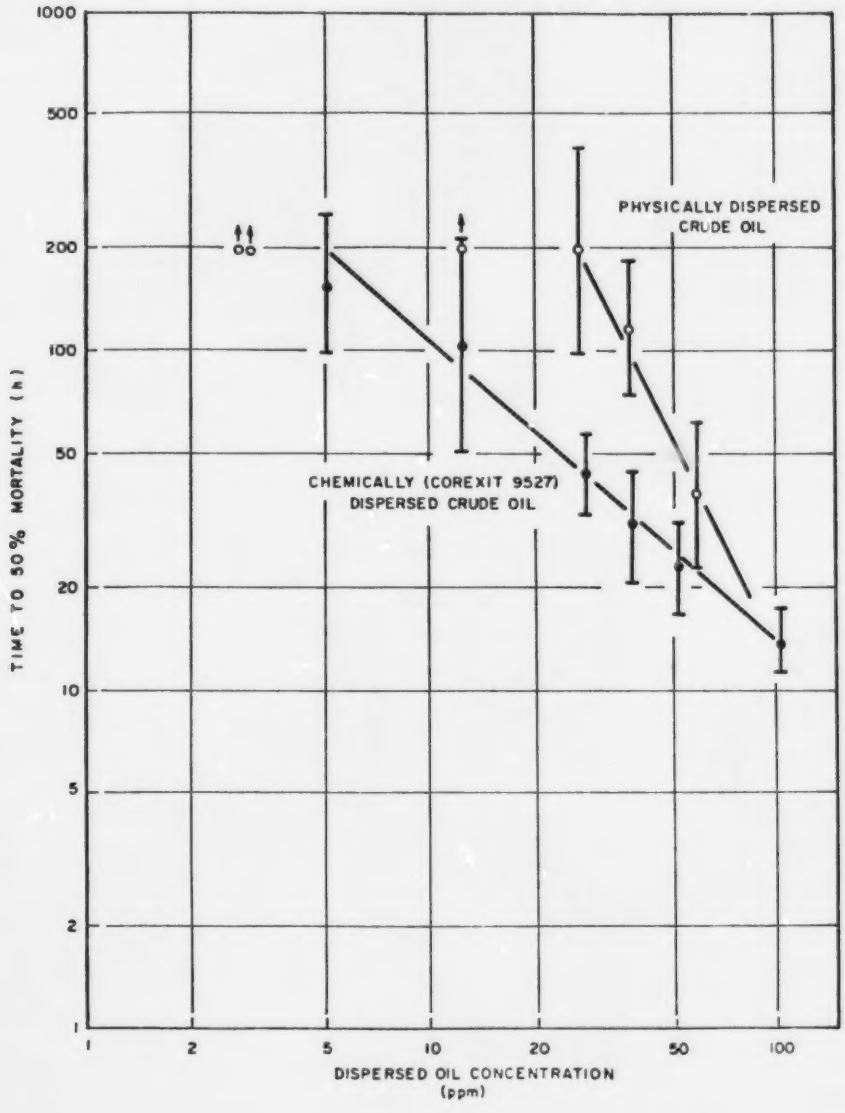

FIGURE 5 TOXICITY CURVES FOR PHYSICALLY AND CHEMICALLY DISPERSED PRUDHOE BAY CRUDE OIL WITH Gnorimosphaeroma oregonensis

TABLE 3 DIFFERENCES IN MORTALITY BY SEX OF Gnorimosphaeroma DURING THE 96-h FLOW-THROUGH BIOASSAY WITH CHEMICALLY DISPERSED PRUDHOE BAY CRUDE OIL

Oil Concentration (ppm)	% Mortality Females	% Mortality Males
5.2	12.5	96.7
12.5	12.0	98.2
28.1	86.0	98.5
38.3	98.1	100.0
51.6	100.0	100.0
101.0	100.0	100.0

The behaviour of treated and control isopods was also monitored during the bioassays. A marked reduction in mating frequency was observed during the early hours of the bioassay with chemically dispersed oil, with decreased mating being more pronounced at higher concentrations as well as during the last day of exposure to lower oil concentrations. In some instances, decreased mating was also observed during exposure of Gnorimosphaeroma to physically dispersed oil, although the concentration and time-related trends were not as clearly evident as with the chemically produced oil-in-water emulsion. Duval and Fink (1980) previously reported decreased mating frequency in this species when individuals were exposed to the water-soluble fraction of Prudhoe Bay crude oil. Consequently, it is possible that the greater effects of chemically dispersed oil on mating are related to the increased opportunity for dissolution of aromatic hydrocarbons when dispersants are added to the crude oil.

Another effect of dispersed oil Gnorimosphaeroma was a loss of coordinated motor ability. At a physically dispersed oil concentration of 59.3 ppm, 98% of the isopods were immobilized after 16 hours; while at 101 ppm of chemically dispersed oil, 90% of the test organisms were immobilized within 2.5 hours. Relative differences in the activity of isopods exposed to physically and chemically dispersed oil are shown in Figure 6. Although it should be emphasized that observations of this type tend to be somewhat subjective and dependent on the time of day that observations are made (i.e. circadian activity rhythms). Time and concentration-related decreases in the general activity of test organisms were nonetheless apparent, particularly with chemically dispersed oil at

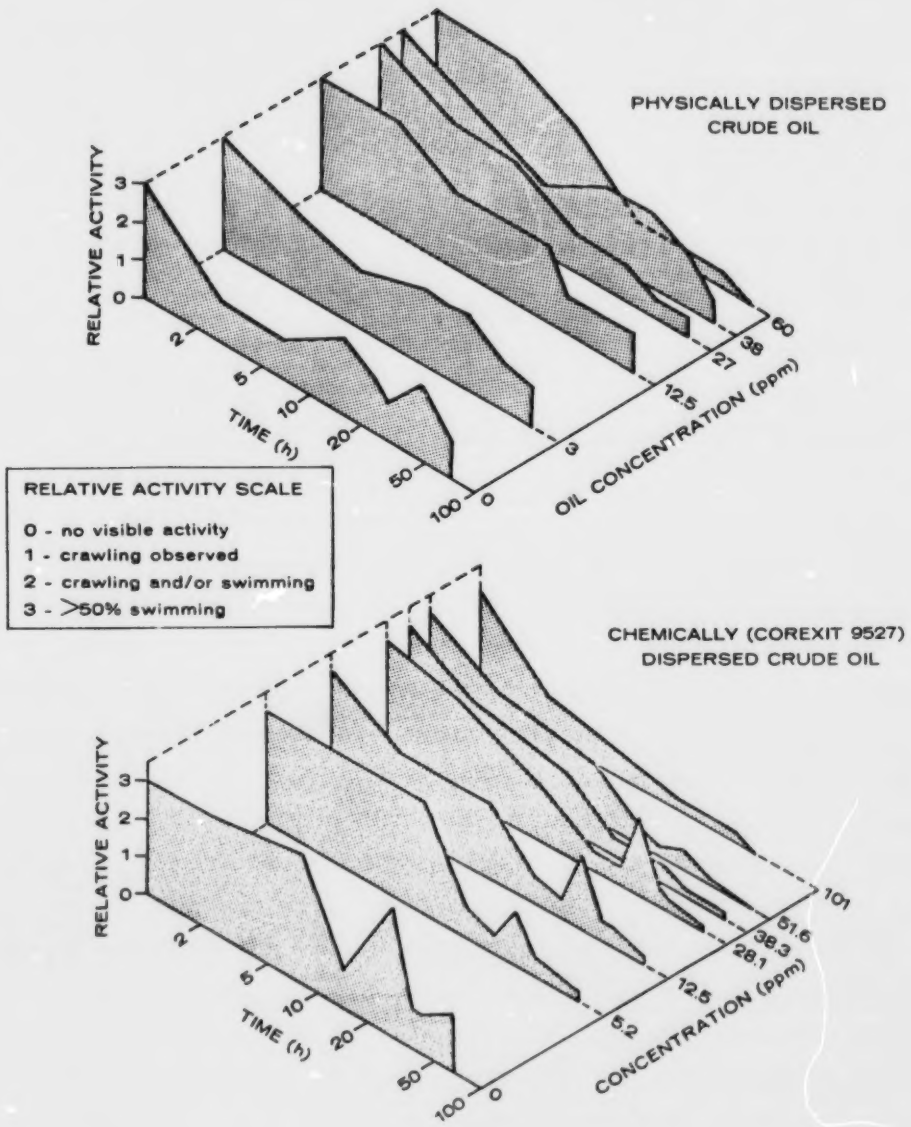

FIGURE 6 RELATIVE DIFFERENCES IN THE ACTIVITY OF Gnorimosphaeroma oregonensis EXPOSED TO PHYSICALLY AND CHEMICALLY DISPERSED PRUDHOE BAY CRUDE OIL

concentrations greater than 28.1 ppm. There were five consecutive stages in the immobilization of isopods following exposure to oil:

1. loss of the ability to crawl in a co-ordinated manner;
2. inability of the organism to right itself when landing on its dorsal surface after swimming;
3. loss of swimming ability;
4. decreased mobility of all appendages except the pleopods; and
5. loss of dorsal-ventral flexing movement (involved in righting response) as well as pleopod activity.

The present studies indicated that recovery from the first three stages of immobilization could occur when isopods were no longer exposed to dispersed oil. However, further studies would be required to confirm the degree and rate of recovery from oil-induced immobilization.

Duval and Fink (1980) reported that exposure of Gnorimosphaeroma to the WSF of Prudhoe Bay crude oil increased moulting. This phenomenon was also examined during the present investigation. Increases in the frequency of moulting, such as those observed in this earlier study, were only found when the isopods were exposed to physically dispersed crude oil, particularly at a concentration of 3 ppm and to a lesser extent 37.9 ppm (see Figure 7). Physically prepared oil dispersions also caused the isopods to moult earlier than normal during the bioassays. On the other hand, exposure of Gnorimosphaeroma to all concentrations of chemically dispersed oil markedly decreased the frequency of moulting (Figure 7), especially when oil concentrations exceeded 12.5 ppm.

The effects of oil on the diel rhythm of body pigmentations and avoidance responses of isopods were also examined during the course of the bioassays. Unlike the altered pigment rhythms observed when this species was exposed to water-soluble hydrocarbons from Prudhoe Bay crude oil (Duval and Fink, 1980), most individuals treated with chemically or physically dispersed oil maintained their normal colouration (light-brown bands during day-light; light translucent brown during darkness) up until the point of death. Avoidance responses to physically dispersed oil were poorly defined and often inconsistent, while exposure to oil dispersed with Corexit 9527 frequently resulted in immediate and strong swimming responses.

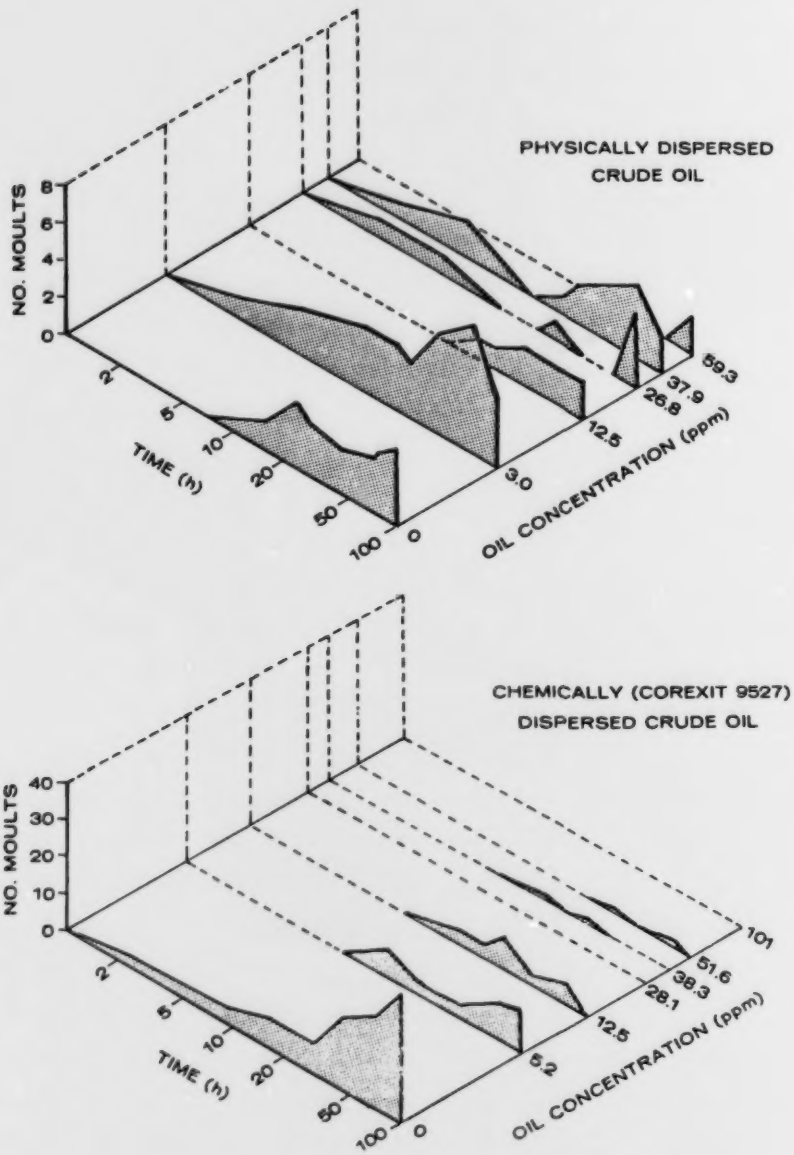

FIGURE 7 DIFFERENCES IN THE NUMBER OF MOULTS PRODUCED BY *Gnorimosphaeroma oregonensis* EXPOSED TO PHYSICALLY AND CHEMICALLY DISPERSED PRUDHOE BAY CRUDE OIL

3.2 Respiration

3.2.1 Experimental Design and Data Analysis. The effects of physically and chemically dispersed oil on respiration of G. oregonensis were studied in eight short-term experiments (Table 1, Section 2.1). Two exposure durations (24- and 48-h) and three concentrations (0, 3, and 20% of the 48-h LC_{50} values) were investigated for each dispersion type. Experiments consisted of a 48-h pre-exposure phase, a 24- or 48-h treatment, and a 48-h recovery or post-exposure phase. Two of the four respirometer vessels were maintained as controls throughout each experiment. Respiration data collected every 30 minutes for organisms in each test vessel were used to calculate 4-h mean rates of oxygen consumption for treated and untreated isopods (see Figures 8 to 11). Mean respiration rates ($\pm\sigma$) of treated and control isopods during each experiment are shown in Tables B to I in the appendix to this report. Comparisons of 4-h mean rates with a Student's t-test were used to determine whether differences in rates were the result of toxicant exposure in any given experiment.

Mean daily rates of oxygen consumption by untreated isopods during the 44 days of measurements ranged from 6.30 to 13.23 $\mu L\ O_2/g$ wet wt/min. The substantial variability in control respiration rates measured during the course of the investigation, both between and within individual experiments (see Table 4), may be related to short-term physiological adjustments to the controlled salinity, light and temperature conditions imposed during the experiments. Normalization of respiration data was necessary, therefore, to facilitate comparison between experiments, particularly for assessment of effects of oil dispersion type, exposure duration and concentration. This normalization was accomplished in the following manner. Using control data from all eight experiments, an overall mean respiration rate was computed for each pre-exposure, exposure, and recovery day in the studies. Normalization factors were then calculated for each day of each experiment by taking the ratio of the observed daily mean control respiration rate and the overall mean rate for the appropriate day number. These factors were applied to respiration rates observed in treatment vessels, to correct for differences in oxygen consumption unrelated to the oil exposure.

3.2.2 General Trends. The predominant feature of respiration in Gnorimosphaeroma oregonensis is a well defined circadian rhythm characterized by elevated evening respiration rates which gradually decrease towards dawn (Duval and Fink, 1980). This daily pattern in oxygen consumption paralleled a general activity rhythm which was characterized by an increase in activity which reached a maximum approximately 3 to 5

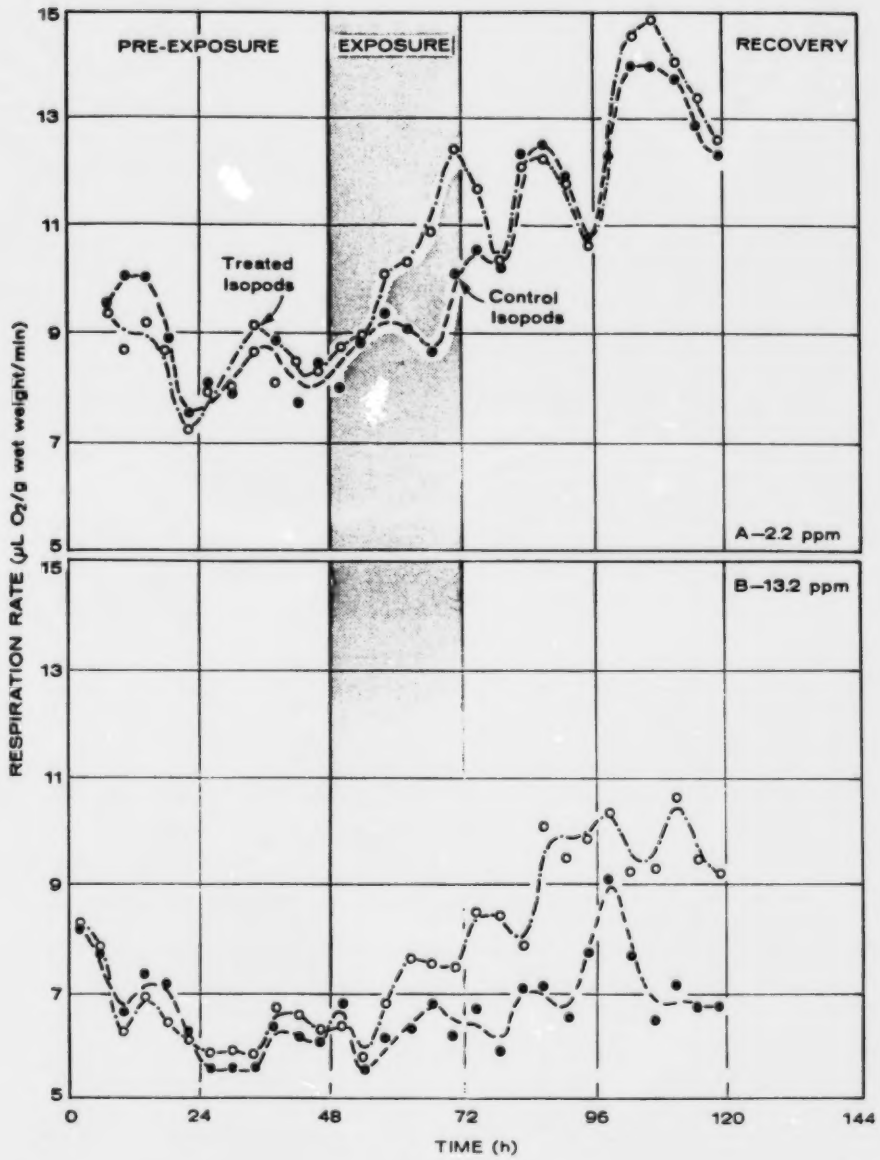

FIGURE 8 THE EFFECTS OF 24 HOUR EXPOSURE TO PHYSICALLY DISPERSED OIL ON THE RESPIRATION OF Gnorimosphaeroma oregonensis

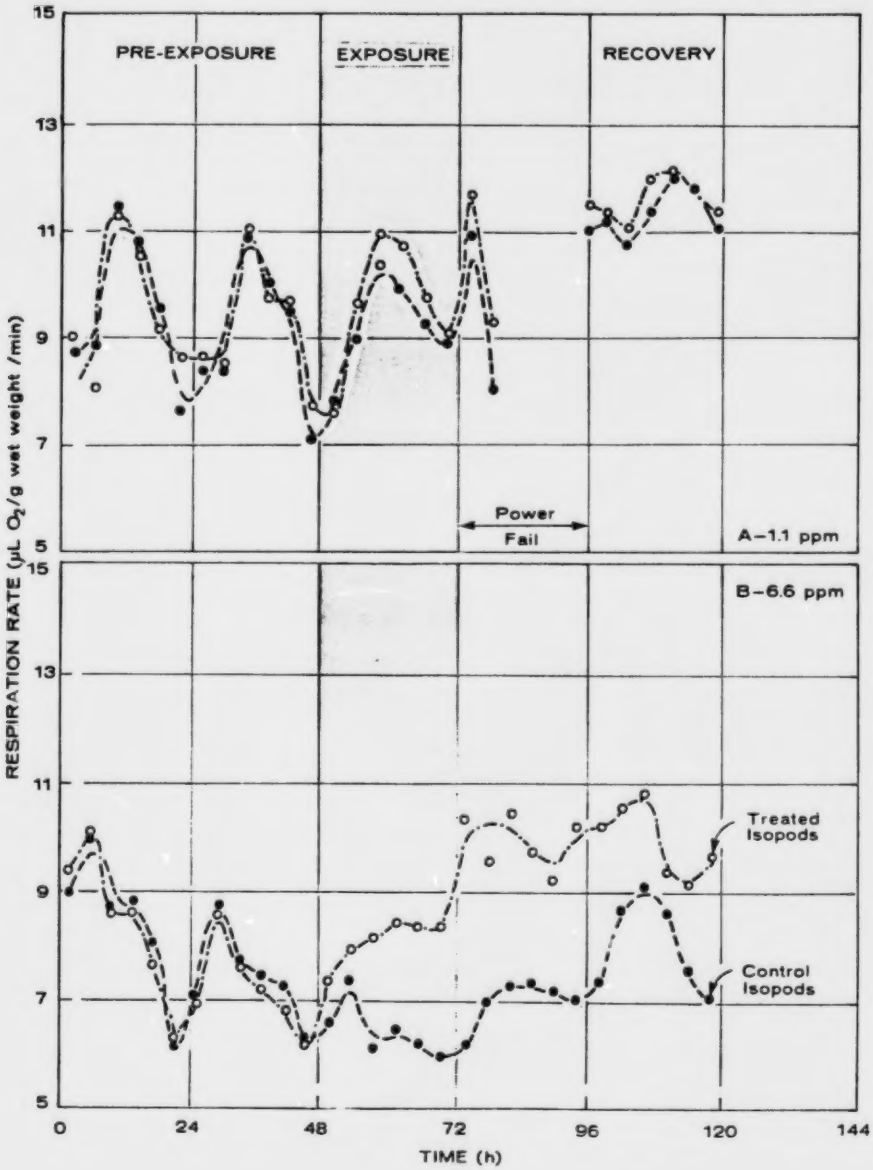

FIGURE 9 THE EFFECTS OF 24 HOUR EXPOSURE OF CHEMICALLY DISPERSED OIL ON THE RESPIRATION OF Gnorimosphaeroma oregonensis

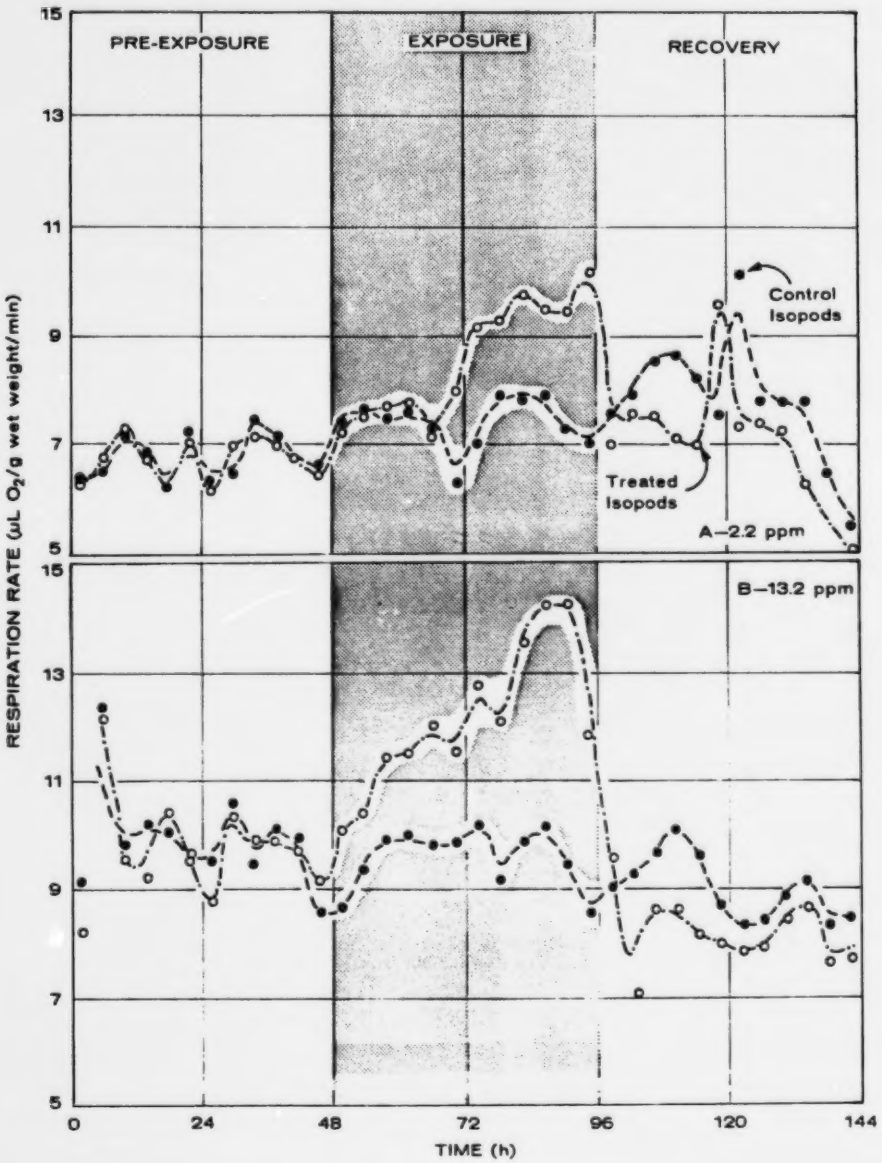

FIGURE 10 THE EFFECTS OF 48 HOUR EXPOSURE TO PHYSICALLY DISPERSED OIL ON THE RESPIRATION OF Gnorimosphaeroma oregonensis

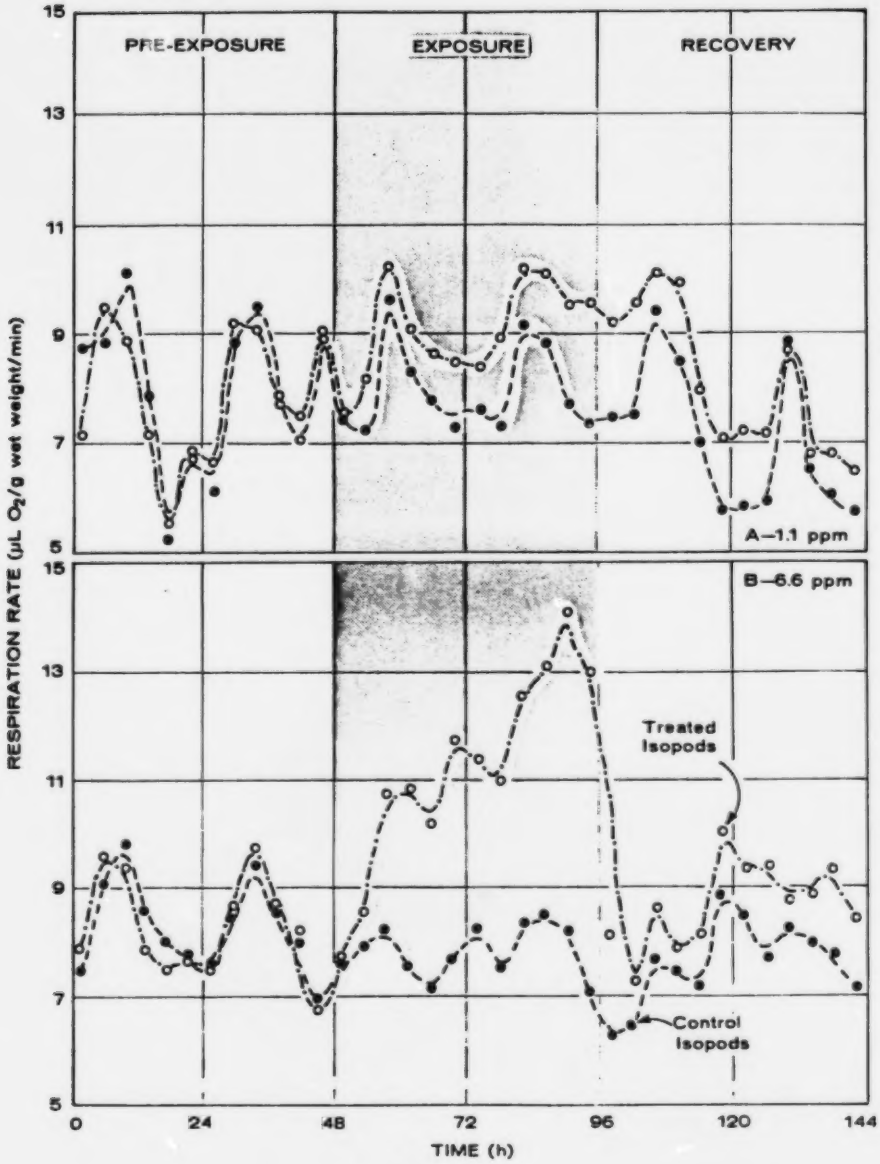

FIGURE 11 THE EFFECTS OF 48 HOUR EXPOSURE TO CHEMICALLY DISPERSED OIL ON THE RESPIRATION OF Gnorimosphaeroma oregonensis

TABLE 4 CHANGES IN THE DAILY MEAN RESPIRATION RATES (µL O_2/g wet wt/min) OF Gnorimosphaeroma IN RESPONSE TO CHEMICALLY (COWD) AND PHYSICALLY (POWD) DISPERSED OIL

Experimental Conditions		Pre-Expos. Day 1	Pre-Expos. Day 2	Exposure Day 1	Recov. Day 1	Recov. Day 2
POWD 2.2 ppm/24 h	C	9.26±2.07	8.28±1.72	9.06±2.30	11.42±2.42	13.25±2.32
	T	8.63±2.28	8.38±2.33	10.26±2.37	11.50±2.68	13.60±2.88
		n=70	n=96	n=96	n=92	n=96
		ns	ns	t=3.560	ns	ns
				***		
POWD 13.2 ppm/24 h	C	7.19±1.37	5.89±0.78	6.30±1.65	6.85±1.86	7.15±1.00
	T	6.93±1.52	6.16±0.62	6.94±1.21	9.05±1.54	9.88±1.01
		n=92	n=96	n=96	n=96	n=96
		ns	ns	t=3.065	t=8.926	t=18.820
				**	***	***
COWD 1.1 ppm/24 h	C	9.50±2.23	9.05±2.16	9.23±2.23	9.87±3.04	11.41±2.43
	T	9.45±2.39	9.21±1.93	9.62±2.34	10.78±3.62	11.57±2.71
		n=90	n=96	n=96	n=32	n=94
		ns	ns	ns	ns	ns
COWD 6.6 ppm/24 h	C	8.34±2.11	7.33±1.73	6.39±1.05	6.88±1.85	8.06±1.83
	T	8.32±2.22	7.15±1.50	8.04±1.14	9.89±2.04	9.94±2.32
		n=94	n=96	n=96	n=96	n=94
		ns	ns	t=10.431	t=10.709	t=6.169
				***	***	***

ns not significantly different at $p>0.05$
* significantly different at $p<0.05$
** significantly different at $p<0.01$
*** significantly different at $p<0.001$

C control
T treated

TABLE 4 CHANGES IN THE DAILY MEAN RESPIRATION RATES (μL O_2/g wet wt/min) OF Gnorimosphaeroma IN RESPONSE TO CHEMICALLY (COWD) AND PHYSICALLY (POWD) DISPERSED OIL (Cont'd)

Experimental Conditions		Pre-Expos. Day 1	Pre-Expos. Day 2	Exposure Day 1	Exposure Day 2	Recov. Day 1	Recov. Day 2
POWD 2.2 ppm/48 h	C	6.67±1.61	6.75±1.57	7.25±1.70	7.49±1.63	8.07±1.61	7.59±1.95
	T	6.68±1.29	6.70±1.13	7.66±1.29	9.16±1.58	7.71±2.25	7.12±1.97
		n=96	n=96	n=96	n=96	n=96	n=96
		ns	ns	ns	t=7.208	ns	ns
						***	
POWD 13.2 ppm/48 h	C	10.19±2.63	9.69±1.87	9.46±2.21	9.64±1.66	9.42±1.97	8.64±2.43
	T	9.87±2.21	9.44±1.47	11.20±1.53	13.20±2.09	8.37±2.13	8.11±1.93
		n=92	n=96	n=96	n=92	n=96	n=96
		ns	ns	t=6.343	t=12.794	t=3.546	ns
				***	***	***	
COWD 1.1 ppm/48 h	C	7.88±2.66	8.02±2.14	7.93±1.98	8.10±1.78	7.60±2.11	6.55±2.13
	T	7.48±2.21	8.16±1.95	8.67±1.78	9.53±1.81	8.92±2.36	7.24±2.26
		n=94	n=96	n=96	n=96	n=96	n=94
		ns	ns	t=2.723	t=5.519	t=4.085	t=2.154
				**	***	***	*
COWD 6.6 ppm/48 h	C	8.42±1.85	8.17±1.34	7.42±1.54	8.01±1.59	7.24±2.05	7.98±1.44
	T	8.19±2.21	8.34±1.11	9.65±2.20	12.44±2.63	8.36±2.40	9.16±1.98
		n=90	n=96	n=96	n=96	n=96	n=96
		ns	ns	t=8.136	t=14.123	t=3.477	t=4.722
				***	***	***	***

ns not significantly different at $p>0.05$
* significantly different at $p<0.05$
** significantly different at $p<0.01$
*** significantly different at $p<0.001$

C control
T treated

hours after dusk. Consequently, throughout this investigation it was necessary to distinguish between changes in oxygen consumption caused by hydrocarbon exposure and daily respiration transients associated with circadian rhythms.

The effects of chemically and physically dispersed oil on the pattern of respiration in Gnorimosphaeroma are illustrated in Figures 8 through 11. In seven of eight experiments conducted, exposure to either chemically or physically dispersed oil produced a significant departure from control respiration rates. Daily mean respiration rates measured in control (C) and treatment (T) vessels for each experiment are indicated in Table 4, together with the results of Student's t-test analyses for the statistical significance of differences between control and treatment rates. The latter analyses demonstrated that respiration rates measured in control and treatment vessels were not significantly different during the pre-exposure phase of all the experiments ($p>0.05$), an important condition which must be met to allow examination of the effects of dispersed oil. The data provided in Figures 8 to 11 also indicate that the diel pattern of oxygen consumption by Gnorimosphaeroma in control and treatment vessels was generally parallel throughout the pre-exposure phase of the experiments.

Exposure of G. oregonensis to both physically and chemically dispersed Prudhoe Bay crude oil resulted in an increase in respiration rate relative to control levels. The degree of deviation from normal respiratory expenditure, however, varied with oil dispersion type, exposure concentration and duration. Major trends observed during the respiration studies were:

1. The deviation from normal rates generally increased with the concentration of both physically and chemically dispersed oil. During 24-h exposures of isopods to physically dispersed oil, rates of oxygen consumption were, on the average, 9.5% and 14.3% higher than normal at 2.2 and 13.2 ppm, respectively. Concentration-related increases in respiration during 24-h treatment with chemically dispersed oil were even larger, averaging 6.8% and 28.0% for 1.1 and 6.6 ppm, respectively.
2. In all cases, increasing exposure duration from 24 to 48 hours caused a progressively larger deviation from normal respiration rates, with OWD concentration continuing to influence the degree of increase in oxygen consumption (see Table 5).
3. Chemically dispersed Prudhoe Bay crude oil had a significantly greater effect on respiration of Gnorimosphaeroma than physically dispersed oil when the exposure concentration of both dispersion types was 20% of the respective 48-h LC_{50}.

TABLE 5 PERCENT CHANGES IN THE DAILY MEAN RESPIRATION RATES OF Gnorimosphaeroma IN RESPONSE TO DISPERSED OIL

Experimental Conditions		Expos. Day 1	Expos. Day 2	Recov. Day 1	Recov. Day 2
POWD	2.2 ppm/24 h	+13.2	-	+ 0.7	+ 2.6
	13.2 ppm/24 h	+10.2	-	+32.1	+38.2
COWD	1.1 ppm/24 h	+ 4.2	-	+ 9.2	+ 1.4
	6.6 ppm/24 h	+25.8	-	+43.8	+23.3
POWD	2.2 ppm/48 h	+ 5.7	+22.3	- 4.5	- 6.2
	13.2 ppm/48 h	+18.7	+36.9	-11.1	- 6.1
COWD	1.1 ppm/48 h	+ 9.3	+17.7	+17.4	+10.5
	6.6 ppm/48 h	+30.0	+55.3	+15.5	+14.8

POWD - physical oil-in-water dispersion
COWD - chemical oil-in-water dispersion

4. The pattern and degree of respiratory recovery following OWD treatment varied with dispersion type, concentration and exposure duration. Recovery following short-term (24-h) exposures to low concentrations of both dispersion types was usually complete during the first 24 hours of the recovery period. Short-term exposure to higher concentrations, as well as longer durations of exposure (48-h) to both low and high concentrations of dispersed oil sometimes resulted in delayed or incomplete respiratory recovery (see Table 5).

3.2.3 24-h Exposure Studies. The respiratory responses of Gnorimosphaeroma during and after 24-h exposures to 2.2 and 13.2 ppm of physically dispersed oil are illustrated in Figures 8A and 8B. At the lower concentration, significant ($p>0.001$) changes in oxygen consumption did not occur until after 16 hours of treatment. For the last 8 hours of exposure, average respiration of treated isopods was approximately 24% higher than control rates. The daily mean rates (Table 4) were only 13.2% higher than normal during the exposure phase of this experiment. Recovery of the treated isopods was very rapid following the end of the exposure period, and after 8 hours, no further differences in oxygen consumption of control and treated isopods were observed. At the higher concentration (13.2 ppm) of physically dispersed crude, the test organisms responded more quickly to the toxicant (Figure 8B), with daily mean respiration rates of treated isopods

being 10.2, 32.1 and 38.2% higher than control rates for the exposure period and the first and second days of recovery, respectively. However, analysis of subsequent respiratory recovery was complicated by methods used during this experiment. At this OWD concentration, it was noted that some oil accumulated in the upper portions of the exposure vessels and was not completely removed by the half hourly purging of the vessels during the post-exposure phase. Therefore, the lack of respiratory recovery following this 24-h exposure may be an artifact of continued exposure to oil. This accumulation of oil was circumvented in subsequent experiments by transferral of test organisms to clean vessels at the end of the exposure period. In some cases, transfer of isopods resulted in a 1 to 2 hour increase in oxygen consumption, although this did not hamper evaluation of recovery responses.

The effects of 24-h exposure of Gnorimosphaeroma to 1.1 and 6.6 ppm of chemically dispersed oil are shown in Figures 9A and 9B. In contrast to physically dispersed oil, the lower concentration (3% 48-h LC_{50}) did not cause any significant difference ($p>0.05$) in the respiration of treated isopods, either during or following the exposure period. At 6.6 ppm, however, a response similar to that resulting from exposure to 13.2 ppm of physically dispersed oil was observed. Respiration rates of treated isopods were significantly higher ($p<0.01$) than normal within 4 hours of the beginning of the exposure period and continued to rise throughout the remainder of the treatment phase. In addition, there was incomplete respiratory recovery during the post-exposure phase. The daily mean respiration rates of treated isopods were 25.8, 43.8 and 23.3% higher than control rates for the exposure period, and the two recovery days, respectively.

3.2.4 48-h Exposure Studies. In general, the effects of dispersed oil on respiration rates observed during the first 24 hours of the 48-h exposure experiments were similar to the results obtained during the 24-h studies. During the second day of the exposure to 2.2 ppm of physically dispersed oil, respiration continued to rise, and on the average was approximately 22% higher than in the control test organisms. The recovery response was somewhat different than that observed following the 24-h exposure, since in this case the respiration of treated isopods decreased to a level less than that of the controls. However, mean daily rates of oxygen consumption by treated and untreated isopods were not significantly different ($p>0.05$; Table 4) on both recovery days, indicating complete respiratory recovery.

The response of Gnorimosphaeroma to 13.2 ppm of physically dispersed oil during the first 24 hours of exposure was consistent with that described earlier (Section

3.2.3). During the second 24-hours of exposure, respiration continued to increase up until 44 hours when it was 50% higher than that of the controls (see Figure 10B). Prior to the end of the exposure period, oxygen consumption of treated isopods began to decline rapidly, and by the 8th hour of recovery, it had decreased from 14.3 to 7.1 μL O_2/g wet wt/min. The initial part of this respiration decrease may have been associated with the normal dawn transient of the circadian rhythm, but the latter portion was almost certainly related to the termination of oil exposure. The daily mean rates of respiration by treated isopods during the first and second day of exposure and the two days of recovery were 18.4 and 36.9% higher than normal on successive exposure days, and 11.1 and 6.1% lower than the controls during recovery. As shown in Table 5, the daily mean oxygen consumption of treated isopods was significantly lower ($p<0.001$) than the control rate on the first day of recovery, but not on the second day, indicating delayed but essentially complete respiratory recovery. The recovery response following this exposure was not directly comparable to that occurring after the 24-h exposure to 13.2 ppm of physically dispersed oil, due to the continued presence of residual oil during the recovery period of the latter study (Section 3.2.3).

The effects of 48-h exposure of Gnorimosphaeroma to 1.1 ppm of chemically dispersed Prudhoe Bay crude oil are shown in Figure 11A. The increase in respiration during the first 24 hours of exposure was slightly greater than that observed during the 24-h exposure study completed at the same concentration, although still only 9.3% higher than the controls on a daily basis (Table 5). As in the case of other 48-h studies, oxygen consumption of treated isopods continued to increase during the second day of exposure to the chemically prepared OWD, and on the average was 17.7% higher than the controls. Although respiration rates remained significantly higher than control levels on the first (17.4%; $p<0.001$) and second (10.5%; $p<0.05$) recovery days, there was evidence of at least partial recovery towards the end of the experiment.

Analysis of the results of the 48-h exposure/6.6 ppm chemically dispersed oil study was complicated by 90% mortality of treated mature male isopods. Although a similar differential mortality of males was observed during the chemically dispersed oil bioassay (Section 3.1; Table 3), analysis of the results of that bioassay indicated that the time to 50% mortality at 6.6 ppm of chemically dispersed oil would be between 100 and 200 hours (Figure 5). This apparent increase in the sensitivity of male isopods to chemically dispersed oil may be related to changes in their physiological condition prior to naturally occurring mortality. No attempt was made to correct respiration rates for this

loss of biomass since the relationship between time into the exposure phase and mortality was unknown. At the same time, the contribution of probable resultant bacterial proliferation to observed respiration rates could not be estimated. Despite this mortality, marked increases (30-55%) in oxygen consumption were observed throughout the exposure phase (Table 5), and although respiration rates of treated isopods began to drop after the 48-h exposure (Figure 11B), they remained significantly higher ($p<0.001$) than control levels (Table 4).

3.2.5 Summary of Respiration Studies. The effects of physically and chemically (Corexit 9527) dispersed crude oil on the respiration rates of Gnorimosphaeroma oregonensis are summarized in Figure 12. These data have been normalized by methods described in Section 3.2.1 to facilitate comparison of different experiments characterized by variable control respiration levels. The results of the present investigation demonstrated that chemically dispersed crude oil had more pronounced and persistent effects on respiration of G. oregonensis than oil which was mechanically dispersed. In general, increasing the exposure duration and concentration increased the degree of departure from normal levels of oxygen consumption. However, with the exception of the 24-h exposure to 13.2 ppm of physically dispersed oil (Section 3.2.3) and the 48-h exposure to 6.6 ppm of chemically dispersed oil, all studies indicated that partial, if not complete respiratory recovery occurred within 48 hours of the end of the treatments.

The effects of petroleum hydrocarbons on respiration rates of marine invertebrates have been examined in a number of previous investigations, although the only studies directed towards isopods have been those of Percy and Mullin (1975) and Duval and Fink (1980). Depending on species, oil type, concentration and exposure duration, respiration rates of isopods have either been stimulated or depressed by exposure to dispersed oil or its water-soluble components. Percy and Mullin (1975) reported 35% and 31% depressions in the oxygen consumption of Mesidotea sibirica during 24-h exposures to dispersions of Norman Wells crude (20-200 ppm) and Pembina crude (10-22 ppm), respectively. On the other hand, the same authors found that respiration of M. entomon increased by 20-68% during exposure to dispersions of Norman Wells crude at concentrations from 10 to 1000 ppm. Duval and Fink (1980) examined the effects of a water-soluble fraction of Prudhoe Bay crude on respiration of the same isopod species used in the present investigation, and found that oxygen consumption either decreased or increased depending on exposure duration and concentration. In the present study, there was no indication of hydrocarbon-related respiratory depression or evidence or reversal of

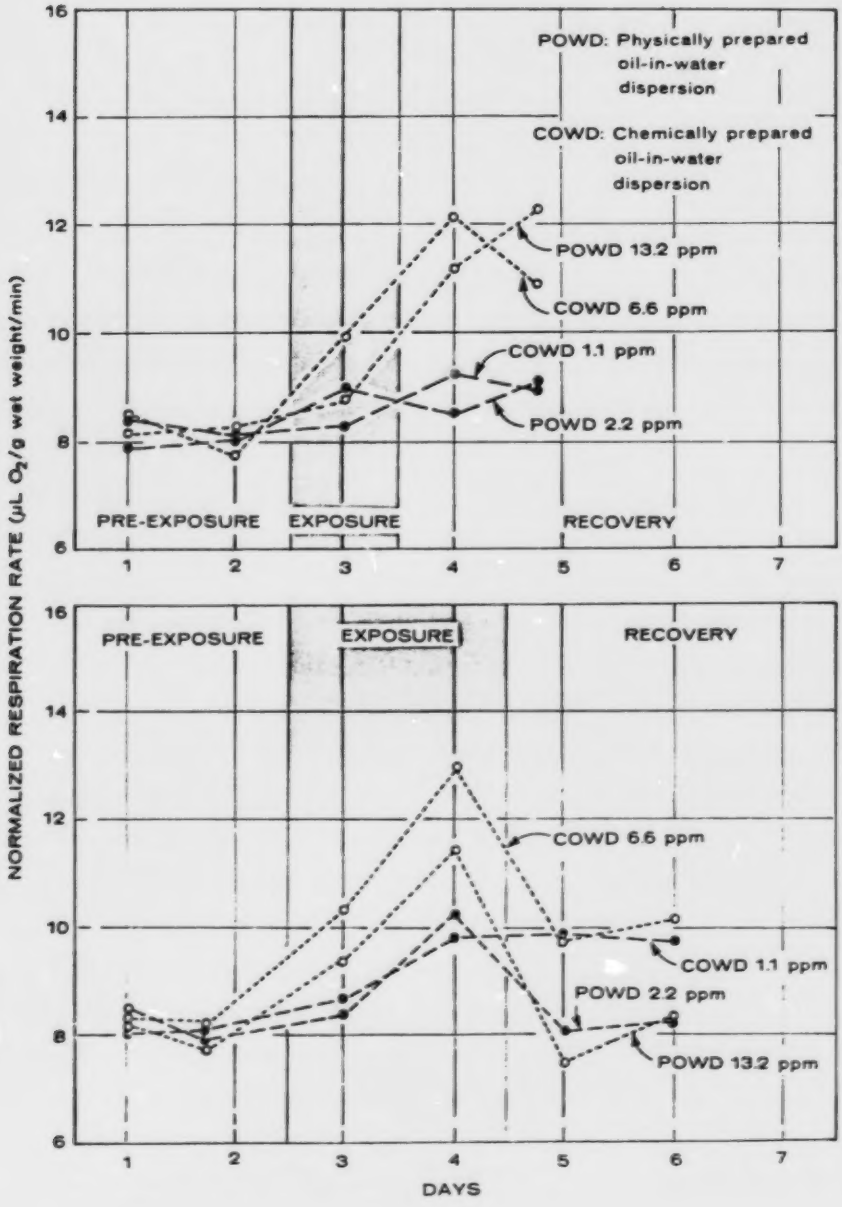

FIGURE 12 SUMMARY OF THE EFFECTS OF PHYSICALLY AND CHEMICALLY (COREXIT 9527) DISPERSED PRUDHOE BAY CRUDE OIL ON THE RESPIRATION RATES OF Gnorimosphaeroma oregonensis

inhibition phenomenon described by Percy and Mullin (1975), despite the fact that our investigations were completed at lower OWD concentrations where respiratory inhibition would be most likely. On the other hand, the enhancement of respiratory metabolism previously documented by Percy and Mullin (1975) was also observed during this study. The relationship between concentration, exposure duration and oxygen consumption of Gnorimosphaeroma lends support to the latter authors' hypothesis that as dispersion concentration increases, certain water-soluble components increase in concentration and penetrate the tissues where they stimulate cellular metabolism. The ecological significance of this and other sublethal effects observed during this investigation are discussed in Section 4.

3.3 Assimilation Rate and Efficiency

Exposure to hydrocarbons can induce changes in behavioural patterns and physiological pathways which may subsequently affect carbon budgets and the amount of energy available for maintenance, growth and reproduction (Gilfillan, 1975). In the present investigation, the effects of sublethal concentrations of physically and chemically dispersed Prudhoe Bay crude oil on carbon assimilation by G. oregonensis were measured in eight short-term studies. Throughout this report, assimilation is taken to mean "net assimilation", or that proportion of the carbon intake of organisms which is converted to new tissue following excretion and respiratory losses. When food intake is constant, net assimilation decreases either when assimilation efficiency decreases, or when energy losses associated with respiratory metabolism or excretion of dissolved metabolites increase.

In general, exposure of isopods, to sublethal amounts of either physically or chemically dispersed crude oil resulted in a significant decrease in carbon assimilation rates and efficiencies when concentrations were approximately 20% of the 48-h LC_{50} and exposure duration was 48 hours. However, effects on assimilation were not apparent after the 48-h recovery period, suggesting that alteration of the carbon budget during oil exposures was temporary.

Two-way analyses of variance were used to test if exposure duration (24-vs. 48-h) and dispersed oil concentration (0, 3 or 20% of 48-h LC_{50}) had a significant effect on assimilation rates (µg C/mg dry wt/day) and assimilation efficiencies (% dpm assimilated per day/dpm fed per day), both before and after the 48-h recovery period. Student's t-tests were subsequently used to determine when significant differences in assimilation rates and efficiencies occurred, as well as the comparative effects of

physically and chemically dispersed oil. As indicated by the data provided in Table 6, there was considerable variability in both assimilation rate and efficiency under equal treatment conditions. This variability was undoubtedly related to the potential for uneven distribution of radiocarbon in the test population since (1) on any given day, all the labelled food introduced was usually consumed by not more than 10% of the test organisms, (2) cannibalism was frequently observed, and (3) food ingested during the post-exposure phase of the experiments did not receive equal time for assimilation. Nevertheless, several trends in radiocarbon uptake were statistically significant and are discussed below.

At the higher concentrations (20% 48-h LC_{50}) of both physically and chemically dispersed Prudhoe Bay crude oil, assimilation rates and efficiencies were significantly lower ($p<0.05$) following the 48-h exposures than after 24-h exposures. Assimilation rates and efficiencies were also significantly lower than control levels immediately after the 48-h exposure to the highest concentration (6.6 ppm) of chemically dispersed oil. On the other hand, 24-h exposure of isopods to both concentrations (2.2 and 13.2 ppm) of physically dispersed oil resulted in a slight but not statistically significant increase in assimilation when compared to control levels. Exposure duration did not have any effects ($p>0.05$) on carbon assimilation at lower concentrations (3% 48-h LC_{50}) of physically or chemically dispersed oil.

The next significant trend with respect to the effects of dispersed oil on the assimilation of carbon by Gnorimosphaeroma was related to oil concentration. The assimilation rate and efficiency of isopods exposed to the higher concentration of chemically dispersed oil (6.6 ppm) for 48 hours were lower than values determined for both control ($p<0.01$) and the lower (1.1 ppm) concentration ($p<0.05$). Oil concentration did not affect radiocarbon assimilation during 24-h exposure to both concentrations of physically dispersed oil.

As indicated by the data provided in Table 6, chemically dispersed oil (6.6 ppm/48-h exposure) caused a greater depression of assimilation rates ($p<0.05$) than the physically prepared OWD (13.2 ppm/48-h exposure), although this difference was no longer apparent at the end of the 48-h recovery period.

Since assimilation efficiency decreased in all situations where assimilation rate was reduced, a decrease in the feeding rate of treated isopods is probably indicated. At the same time, the significant increase in respiratory expenditure observed during 48-h exposures to the higher (20% LC_{50}) oil concentrations (Section 3.2.4), may have also

TABLE 6 CARBON ASSIMILATION BY THE ISOPOD, Gnorimosphaeroma oregonensis, FOLLOWING EXPOSURE TO SUBLETHAL CONCENTRATIONS OF PHYSICALLY AND CHEMICALLY DISPERSED PRUDHOE BAY CRUDE OIL ($X \pm \sigma$)

	Oil Concentration (ppm)	Exposure Duration (h)	Assimilation Rate (µg C/mg dry wt/day)			Assimilation Efficiency (%)		
			Control (n=32)	Before Recovery (n=8)	After Recovery (n=8)	Control (n=32)	Before Recovery (n=8)	After Recovery (n=8)
Physically Dispersed Oil	2.2	24	0.172±0.21	0.264±0.3	0.137±0.11	25.62±23.2	20.0±24.4	21.6±18.1
		48	0.139±0.18	0.075±0.1	0.129±0.18	19.60±22.0	8.9±13.4	25.4±38.1
	13.2	24	0.172±0.21	0.266±0.23	0.080±0.07	25.62±23.2	62.5±57.4	18.7±18.2
		48	0.139±0.18	0.077±0.03	0.022±0.014	19.60±22.0	10.7±5. 0	4.3± 2.9
Chemically Dispersed Oil	1.1	24	0.316±0.34	0.184±1.22	0.178±0.158	58.2±64.0	33.0±25.3	33.9±32.3
		48	0.139±0.11	0.088±0.055	0.159±0.11	43.2±51.2	25.4±17.1	30.2±22.5
	6.6	24	0.316±0.34	0.114±0.095	0.187±0.10	58.2±64.0	19.5±17.4	32.0±18.5
		48	0.139±0.11	0.040±0.017	0.155±0.266	43.2±51.2	4.8 ± 2.6	27.2±49.9

contributed to a reduction in net carbon assimilation. Although not examined in the present investigation, increased excretion such as that observed by Duval and Fink (1980) may have also affected carbon flux in treated isopods. Nevertheless, this study has indicated that despite apparent decreases or increases in carbon assimilation rates and efficiencies due to hydrocarbon exposure, such effects were of relatively short duration. Mean assimilation rates and efficiencies measured after the 48-h recovery phase of our studies were never significantly different from control levels.

Although other studies have examined the effects of water-soluble hydrocarbons on carbon assimilation in marine invertebrates, the effects of sublethal levels of physically and chemically dispersed crude oil on isopods have not been previously documented. Duval and Fink (1980) described the sublethal effects of a WSF of Prudhoe Bay crude oil on the same isopod species used in the present study. These authors reported that low concentrations (2.4 ppm) of water-accommodated hydrocarbons slightly increased carbon assimilation, while higher oil concentrations (9.7 ppm) clearly decreased assimilation rate as exposure duration increased. The latter trend was attributed to the marked increase in the respiratory expenditure of isopods at this concentration.

Other investigations of the effects of petroleum hydrocarbons on carbon flux in crustaceans and molluscs are also consistent with results of the present study. For example, Gilfillan (1975) demonstrated that a 1 ppm WSF of Mid-Continent Sweet Crudes reduced feeding and assimilation, and increased respiration in two species of mussels (Mytilus edulis, Modiolus demissus). Gilfillan et al. (1976) subsequently measured carbon flux in clams (Mya arenaria) following exposure to No. 6 fuel oil from the tanker "Tamano". These authors reported that Mya in contaminated areas showed significantly lower rates of net carbon flux and growth due to decreased feeding rates and increased respiratory expenditure. Milovidova (1974, cited in Johnson, 1977) also reported reduced feeding rates in isopods (Idotea baltica basteri) exposed to 1 000 ppm (initial oil concentration) of Anastasiyevka crude, while Wells and Sprague (1976) demonstrated a reduction in food consumption by lobster larvae (Homarus americanus) during 30-day exposures to 0.11 ppm of Venezuelan crude.

The results of this investigation suggest that depression of carbon assimilation in Gnorimosphaeroma may be more a function of exposure duration than oil concentration, and that chemically dispersed oil may have a more pronounced short-term sublethal effect on carbon assimilation than physically dispersed oil. However, such effects are likely of relatively short duration.

3.4 Naphthalene Uptake and Depuration

The concentration of naphthalenes in the tissue of the isopod, Gnorimosphaeroma oregonensis was determined following exposure to sublethal amounts of physically and chemically dispersed Prudhoe Bay crude oil, both immediately after treatment and after a 48-h recovery period. The results of these analyses are summarized in Table 7. The following general trends were observed:

1. isopods exposed to either type of OWD accumulated naphthalenes in their tissues;
2. there was little or no evidence to suggest that naphthalenes were depurated during the 48-h recovery period;
3. increased concentration of both oil dispersion types caused a general increase in uptake of naphthalenes;
4. exposure duration had no significant effect on uptake of naphthalenes; and
5. accumulation of naphthalenes was more pronounced in test organisms exposed to chemically dispersed oil than physically dispersed oil.

TABLE 7 CONCENTRATIONS OF RESIDUAL NAPHTHALENES IN TISSUES OF THE ESTUARINE ISOPOD, Gnorimosphaeroma oregonensis FOLLOWING EXPOSURE TO PHYSICALLY AND CHEMICALLY (COREXIT 9527) DISPERSED PRUDHOE BAY CRUDE OIL (Mean ± σ indicated)

Dispersion Type	Exposure Concentration (ppm)	Tissue Naphthalenes (ppm) Control n=8		Post-Exposure n=4		Post-Recovery n=4	
Physical	2.2	1.26	0.85	2.55	2.11	2.33	1.00
	13.2	1.19	0.55	8.45	6.87	5.78	1.08
Chemical	1.1	2.50	1.40	5.73	2.43	7.73	1.77
	6.6	1.49	0.86	9.68	1.26	11.30	6.67

The uptake of naphthalenes by marine invertebrates exposed to an oil-in-water dispersion or water-soluble fraction of crude oil has been well documented. However, no studies have examined the uptake and subsequent depuration of hydrocarbons in isopods exposed to physically and/or chemically dispersed crude oil. Duval and Fink (1980) reported that tissue naphthalene levels in the isopod, G. oregonensis were twice the

control levels following 24- to 48-h exposures to a Prudhoe Bay crude WSF (2.4 ppm) and a 48-h period for depuration. These findings are consistent with those of the present investigation since no evidence of naphthalene depuration by isopods was observed 48 hours after treatment with chemically dispersed oil, and insignificant depuration of hydrocarbons from test organisms exposed to physically dispersed oil. In contrast, amphipods (Anisogammarus confervicolus) exposed to a 2.2 ppm WSF for 24 hours had tissue naphthalene concentrations which were not significantly different from control levels after a 48-h recovery period (Duval and Fink, 1980). The authors suggested that amphipods had probably accumulated hydrocarbons, but unlike the isopods tested, naphthalenes were almost completely depurated from tissues during the 48-h recovery phase of the studies.

Other studies have examined the uptake and depuration of petroleum hydrocarbons by decapod crustacea. For example, Anderson (1975) (cited in Varanasi and Malins, 1977) found that levels of naphthalenes in the tissue of grass shrimp (Palaemonetes sp.) exposed to an OWD (20-h) and WSF (24-h) of No. 2 fuel oil reached a maximum level after 6-7 hours, and then gradually decreased during the remaining hours of exposure. When the shrimp were subsequently placed in oil-free water, depuration of hydrocarbons continued and was virtually complete in 48 hours. Anderson et al. (1974) also conducted an investigation of uptake of naphthalenes in brown shrimp, Penaeus aztecus during a 30 minute exposure to a 30% WSF of No. 2 fuel oil. The test organisms accumulated naphthalenes to concentrations which were 10 times higher than present in the test media. However, when the shrimp were subsequently placed in clean water, naphthalenes were gradually discharged over a period of 10 hours.

In the present investigation, two-way analyses of variance and Students' t-tests were used to determine if exposure duration (24- vs 48-h) and concentration (0, 3 and 20% 48-h LC_{50}) of physically and chemically dispersed oil had a significant effect on tissue naphthalene levels in Gnorimosphaeroma, both immediately after treatment and after a 48-h recovery period. As indicated earlier, exposure duration did not have an effect on uptake of naphthalenes. Consequently, data for both 24-h and 48-h exposures were combined to increase sample replication to properly assess concentration effects and differences in uptake of naphthalenes associated with dispersion type. Exposure of isopods to physically dispersed oil at a concentration of 2.2 ppm did not result in significant uptake of naphthalenes ($p>0.05$). However, exposure to both concentrations of chemically dispersed oil and the higher concentration (13.2 ppm) of physically dispersed

crude caused marked accumulation of naphthalenes ($p<0.01$). Concentration also had a significant effect ($p<0.05$) on the degree of naphthalene uptake in the case of both physically and chemically dispersed oil. As indicated in Table 7, there may have been some depuration of hydrocarbons from isopods exposed to the physically dispersed crude oil in the 48 hours following treatment, although this decrease in naphthalenes was not statistically significant ($p>0.05$). On the other hand, there was a tendency for naphthalene levels to be slightly (but not significantly) higher at the end of the 48-h recovery phase than immediately after treatment with the chemically prepared oil-in-water emulsion. The reason for this trend is not clear, but may be related to biological variability or the much smaller oil particle size and greater opportunity for contact with test organisms when Corexit 9527 was used to disperse the Prudhoe Bay crude. Since these dispersions remain in suspension for longer periods and were comprised of smaller particles, they may have adhered to the isopods or become entrapped in crevices on the body surface, causing a potential for further uptake of naphthalenes after the end of the actual exposure phase.

The results of this investigation are consistent with previous studies completed by authors with the WSF of Prudhoe Bay crude oil and Gnorimosphaeroma, with both investigations suggesting that depuration of naphthalenes from this species requires more than 48 hours. The design of the present study did not allow clear definition of the time necessary for depuration of hydrocarbons, although it was almost certainly longer than periods documented for shrimp (Anderson et al., 1974; Anderson, 1975) and amphipods (Duval and Fink, 1980). In the long-term studies described in greater detail in Section 3.6, isopods were treated with 18 ppm of physically dispersed oil or 9 ppm of chemically dispersed oil for 24 hours. Eight days after this exposure, naphthalene levels in tissues were not significantly different from control levels. This suggests that complete depuration of hydrocarbons by Gnorimosphaeroma probably required somewhere between two and eight days.

The present results also suggest that exposure to chemically dispersed oil results in greater uptake of hydrocarbons than exposure to physically dispersed oil. This may be attributed to the greater oil surface to volume ratio achieved when Corexit 9527 was used, likely increasing the concentration of soluble hydrocarbons in the water column and the opportunity for uptake by the test organisms.

3.5 Behavioural Responses to Sublethal Oil Exposure

The effects of physically and chemically dispersed oil on behaviour of Gnorimosphaeroma were investigated during the bioassays and short-term sublethal

investigations. In both types of studies, daily records of behaviour were maintained to identify any changes which may be associated with oil exposure. Pronounced effects on moulting and mating or pairing responses were observed during the bioassays and were previously discussed in Section 3.1. Similar effects were observed during the short-term experiments; however, alterations in moulting and mating were only evident during the 48-h exposures to 6.6 ppm and 13.2 ppm of chemically and physically dispersed oil, respectively. In the chemically dispersed oil, the normal pairing response was virtually eliminated, and most males lost their characteristic diel rhythm in external pigmentation. It should be noted, however, that high mortality of males was also observed in this study (Section 3.2.4). At the same time, a reduction in moulting frequency was evident during the 48-h exposure to 6.6 ppm of chemically dispersed oil. During the recovery period of this study, body pigmentation of survivors appeared to return to normal, although pairing responses and moulting frequencies did not. Exposure to 13.2 ppm of physically dispersed oil for 48 hours resulted in a reduction in frequency of moulting but no other behavioural changes. Ingestion and decay of moults prevented analysis of the return of the moulting behaviour during the post-treatment phase of this investigation.

Alteration of normal behavioural patterns of Gnorimosphaeroma in the presence of hydrocarbons probably occurs in a progressive sequence, with moulting frequency being affected before either pairing responses or pigmentation. The results of the present investigation also indicated that chemically dispersed oil had a greater effect on isopod behaviour than comparable concentrations of physically dispersed oil, since both mating and colouration were affected in addition to moulting frequency with the former oil type. The generally more pronounced sublethal effects of chemically dispersed oil on Gnorimosphaeroma were also evident in terms of respiration, carbon assimilation and uptake of naphthalenes.

The effects of sublethal and chronic exposure to petroleum hydrocarbons on isopod behaviour were previously described by Duval and Fink (1980) and Percy (1978). The former authors examined alteration of moulting frequency, pairing responses, and pigmentation in isopods (G. oregonensis) exposed to a WSF of Prudhoe Bay crude oil. All concentrations tested (2.4-9.7 ppm) caused an increase in moulting frequency, while 5.2 and 9.7 ppm resulted in an inhibition of reproductive behaviour and loss of normal pigmentation. With the exception of increased moulting, these changes in behaviour are consistent with the findings of the present investigation. Differences between the toxicants used (WSF vs. dispersed oil) in the two studies may have influenced the observed

effects on moulting, although the timing of the investigations in view of seasonal differences in the moulting cycle may also have contributed to the variable response to crude oil. Percy (1978) monitored the onset of the moulting cycle and duration of the intermoult period in juvenile isopods (Mesidotea entomon) during chronic exposures to a WSF of Norman Wells and Pembina crudes. He reported that changes in the moult cycle only occurred at oil concentrations near the chronic lethal level. An increase in the duration of the intermoult period was observed at 1.12 ppm of weathered crude, although subsequent intermoult periods were reduced by approximately half after each moult. Percy (1978) suggested that this phenomenon may have represented a physiological adaptation of isopods to oil with time. It is difficult to compare Percy's results to the present findings because exposure duration and oil types varied. Nevertheless, in both investigations, exposure to hydrocarbons did have an effect on frequency of moulting in isopods.

In addition to the avoidance response previously noted with chemically dispersed oil (Section 3.1), exposure to high concentrations of physically and chemically dispersed oil appeared to inhibit the normal response of the isopods to begin swimming upon detection of an air/water interface. This response may be related to the observed preference of Gnorimosphaeroma to remain submerged throughout the tidal cycle, and loss or inhibition of this behavioural pattern could affect survival of this species following an oil spill. The ecological significance of alteration of this and other behavioural responses due to hydrocarbon exposure are discussed in Section 4.

3.6 Long-term Effects of Short-term Oil Exposure

The long-term effects of 24-h exposure of G. oregonensis to physically and chemically dispersed oil were examined in a flow-through system described in Section 2.6. Test organisms were treated with physically (18 ppm) or chemically (9 ppm) dispersed oil at concentrations approximating 27% of 48-h LC_{50}, and subsequently maintained for 8 weeks in oil-free seawater. Parameters measured during this period included frequency of moulting, number of emergent juveniles, and adult mortality, as well as body length, weight and residual naphthalenes in tissues of a subsample of isopods removed from the vessels each week.

Near the end of the 24-h exposure period, an estimated 10% of the male isopods treated with chemically dispersed oil were observed lying on their dorsal surface on the bottom of the vessels. These individuals were presumably unable to right themselves, even though appendage mobility did not appear to be impaired. Such effects

were not observed in isopods exposed to the physically dispersed oil. At the same time, mortality of isopods was negligible (2 to 3%) under both treatments, as well as in the untreated controls. The subsequent 8 week monitoring program indicated that the short-term oil exposures did not have any significant long-term effects on tissue naphthalene levels (measured after 8 days), growth, reproductive success or the survival of adult isopods. Sublethal effects which may have become more apparent after 8 weeks, such as survival of juveniles or longevity of adults, were not examined during the present investigation.

3.6.1 Tissue Naphthalenes. A weekly analysis of the level of naphthalenes in tissues of treated and untreated isopods was completed throughout the long-term studies. Results from the first analysis conducted 8 days after treatment and in all subsequent weeks indicated that levels of naphthalenes in tissues of isopods were not significantly higher than control levels (see Appendix, Table J). Since there was no significant depuration of tissue hydrocarbons after the 48-h recovery period of the short-term studies (Section 3.4), it is speculated that Gnorimosphaeroma required between 2 and 8 days to depurate naphthalenes accumulated during short-term exposures to sublethal concentrations of oil.

3.6.2 Growth and Moulting. Growth reflects the product of interacting physiological and behavioural functions, and can be a useful indicator of stress in some organisms. Growth parameters measured in adult isopods during this study were total body length and weight (Appendix, Table K). In addition, frequency of moulting was also recorded since it is a process closely related to growth in crustacea, and an increase in sensitivity to environmental stress is associated with the moulting cycle in this taxonomic group (Swedmark, 1971; Lockwood, 1967).

A two-way analysis of variance was used to determine if body length of treated isopods was significantly different from untreated controls during each week of long-term study. Exposures to either physically or chemically dispersed oil had no significant effect on body length ($p>0.05$). There were also no significant changes in body length within any given treatment over time. The average length of female and male Gnorimosphaeroma during the 8-week study was 7.9 mm and 5.1 mm, respectively. In addition, the dry weight of isopods was unaffected by oil exposures ($p>0.05$), with the mean dry weight of females and males being 23.03 mg and 6.95 mg, respectively. No differences in the frequency of moulting were found between control and exposed isopods

throughout the long-term study (see Appendix, Table K). Moulting frequency was high (+40%) during the first week after exposure, and low during the remainder of the investigation.

It should be emphasized, however, that the lack of significant effects of OWD exposure on growth and moulting of Gnorimosphaeroma may also be in part related to the timing of the study which coincided with the reproductive life history phase of this species. Since there was no significant increase in body weight during the 8-week study and juveniles were present in laboratory and field populations during the experimental period, it is possible that energy normally directed towards growth was used in reproduction. The almost complete mortality of male Gnorimosphaeroma observed during the period from mid-May to June also lends support to the hypothesis that a greater proportion of energy was directed towards processes other than growth.

Although the effects of petroleum hydrocarbons on growth and moulting patterns of crustacea have been examined in previous investigations, the majority of these studies were focused on the effects of chronic oil exposure on larval or juvenile invertebrates, rather than the short-term exposure of adults such as was involved in this study. For example, Percy (1978), exposed juvenile arctic marine isopods (Mesidotea entomon) to the WSF of weathered and unweathered crude for 160 days and demonstrated only slight effects on growth and moulting at concentrations lower than those which were lethal during chronic exposure. Cox and Anderson (1973) also reported no significant effect on growth of juvenile brown shrimp (Palaemonetes aztecus). On the other hand, Tatem (1976) found reduced growth of larval grass shrimp (P. pugio) following exposure to a WSF of No. 2 fuel oil for 12 days. Clearly, there is considerable variability in the documented effects of petroleum hydrocarbons on crustacea depending on exposure conditions and species. However, larval and juvenile stages are generally more sensitive than adults (Linden, 1976; Percy, 1978; Broderson, 1977). The results of the present investigation are not directly comparable with past studies, but nevertheless they suggest that relatively short-term exposure of adult G. oregonensis to sublethal concentrations of dispersed oil has no apparent effect on growth. Further studies would be required to assess the effects of longer exposures or potential changes in growth at other times of the year.

3.6.3 Reproductive Success. Reproduction in marine crustacea is in part dependent upon exogenous chemical signals, and disrupted reception of these signals by low levels of hydrocarbons in the environment could alter reproductive success (Johnson, 1977). Changes in behaviour in response to oil (e.g. avoidance) could also reduce reproductive

success by displacing mating activities in space and time. The parameter of reproductive success investigated during this study was the number of emergent juveniles observed on a weekly basis in each vessel after their first appearance four weeks after the initiation of the long-term studies. Results are expressed as the weekly number of juveniles per female present in week four (Appendix, Table L), since weekly sub-sampling of isopods without replacement prevented calculation of the number of young per female present in any given week. In addition to monitoring reproductive success of the three test populations, records were maintained of life history stages present in the isopod population in the Squamish River estuary. However, interpretation of the results of this aspect of the research program was limited by the weekly removal of females (potentially gravid) and the lack of available information on the life cycle of Gnorimosphaeroma oregonensis.

Following fertilization, developing embryos are held in the brood pouch of the female until their subsequent emergence as juveniles. The first emergent juveniles in both field and laboratory populations were observed in late May, approximately 4 weeks after oil exposure in the laboratory. Since the gestation period for G. oregonensis probably ranges from 2 to 6 weeks (Hoestlandt, 1973), the treatment phase of the long-term studies could have occurred at the same time as fertilization and/or embryonic development in some or all of test organisms. In any event, exposure to either physically or chemically dispersed oil did not change the time of juvenile emergence with respect to either untreated controls or the natural population. At the same time, analysis of the difference between slopes of the functions describing the relationship between number of juveniles and time (Sokal and Rohlf, 1969) indicated no significant difference in the number of emergent juveniles between each treatment (physically and chemically dispersed oil) and the control population. In all cases, the number of juveniles per female steadily increased for two weeks after their first appearance and then remained relatively constant.

The effects of petroleum hydrocarbons on the reproductive success of crustaceans have been well documented. However, again direct comparison with the present study is difficult because of the differences in experimental design of various investigations. In general, most authors report that adult reproductive success is reduced by hydrocarbon exposure. For example, Tatem (1976) reported that a 72-h exposure of gravid grass shrimp (P. pugio) to a 1.44 ppm WSF of No. 2 fuel oil caused a marked reduction in fecundity. Treated females produced an average of 9 larve compared to an average of 45 larvae from control females. Linden (1976) also demonstrated a reduction

in fecundity and an inhibition of the precopula stage in amphipods (Gammarus oceanicus) during a chronic (60 day) exposure to crude oil.

In summary, decreased reproductive success following chronic oil exposure has been documented in crustacea other than isopods. However, the results of the present investigation suggest that reproductive success of Gnorimosphaeroma was not affected by a 24-h exposure to physically or chemically dispersed oil at concentrations approximating 27% of the 48-h LC_{50}. This apparent difference in sensitivity may be related to the relatively short duration of oil exposure in the present investigation, or the relatively resistant nature of adult isopods (Percy and Mullin, 1975).

3.6.4 Adult Survival. Survival of adult isopods during the 8-week long-term study was calculated as percent mortality of the males and females remaining every week following each treatment (Appendix, Table M), since weekly subsampling without replacement prevented determination of cumulative mortality. Student's t-tests were used to compare percent mortality between treatments and the control population. These analyses indicated that short-term exposure to both physically and chemically dispersed oil did not have a significant effect on survival of adult Gnorimosphaeroma. The average weekly mortality of isopods following both treatments and in the untreated controls was 3.2% and 27% for females and males, respectively. Since the higher mortality of males ($p<0.01$) was observed in both treated and untreated populations, this was assumed to be a natural phenomenon and unrelated to hydrocarbon exposure. Higher mortality of males in the natural Squamish River estuary population also lends support to this conclusion.

The long-term mortality of isopods following short-term exposure to dispersed oil has not been previously examined. However, in a study similar in design to the present investigation, Linden (1976) documented reduced survival of adult amphipods (Gammarus oceanicus) following exposure to 100 and 300 ppm of Venezuelan crude for 48 hours. Both concentrations caused high mortality in amphipods during the second week of the recovery phase. Delayed mortality of this type was not observed during the present investigation with the isopod Gnorimosphaeroma. As indicated earlier, Percy and Mullin (1975) investigated the effects of short-term oil exposures on several arctic marine isopods and concluded that adults were very resistant to oil.

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APPENDIX

DATA ACCUMULATED FROM THE STUDY

TABLE A PERCENT MORTALITY OF Gnorimosphaeroma OBSERVED DURING 96-h FLOW-THROUGH BIOASSAYS WITH PHYSICALLY AND CHEMICALLY DISPERSED PRUDHOE BAY CRUDE OIL

A. Physically Dispersed Prudhoe Bay Crude Oil - Estimates of Percent Mortality

	Dispersed Oil Concentration (ppm)*							
Time (h)	0.0	0.0	2.8	3.0	12.5	26.8	37.9	59.3
1.5	0	1	1	1	0	0	0	1
3.0	0	1	1	4	1	2	1	5
7.0	0	1	1	4	1	2	1	5
16.0	0	2	1	4	1	2	1	14
25.0	0	2	1	4	1	3	2	29
30.0	0	2	1	4	1	5	2	29
47.0	3	2	2	4	1	14	4	57
73.0**	3	2	3	8	3	19	28	90
96.0**	3	2	6	18	20	44	51	100

B. Chemically (Corexit 9527) Dispersed Prudhoe Bay Crude Oil

	Dispersed Oil Concentration (ppm)*							
Time (h)	0.0	0.0	5.2	12.5	28.1	38.3	51.6	101
2.5	0	1	0	0	0	0	0	3
9.0	0	1	0	0	0	0	10	14
16.0	0	1	0	0	0	5	13	50
24.0	1	1	0	0	18	20	50	70
31.0	1	1	4	20	18	80	75	90
45.0	1	1	4	20	35	80	75	90
72.0**	1	2	20	52	90	90	96	100
96.0**	1	2	39	52	94	99	100	100

* Mean oil concentration measured with Coulter Counter.
** Actual mortality after direct count.

TABLE B 4-h MEAN RESPIRATION RATES (± σ) OF Gnorimosphaeroma oregonensis (μL O_2/g wet wt/min)

Experiment P-3: Physically Dispersed Prudhoe Bay Crude Oil: 24-h Exposure at 2.2 ppm (see Figure 8A)

Day	Time	Control	Treatment
Pe1	1800	9.40±2.09	9.54±2.44
	2000	10.16±1.66	8.74±2.00*
	2400	10.18±1.19	9.18±1.93
	0400	8.92±1.07	8.75±1.73
	0800	7.51±2.97	7.25±3.00
Pe2	1200	8.03±1.90	7.98±2.51
	1600	7.85±1.33	7.96±2.10
	2000	8.65±1.72	9.18±2.36
	2400	8.88±1.68	8.16±2.12
	0400	7.68±1.39	8.48±1.96
	0800	8.56±2.10	8.39±2.96
Exp1	1200	8.01±2.80	8.76±2.08
	1600	8.89±1.66	8.94±2.03
	2000	9.44±1.70	10.14±1.90
	2400	9.15±1.85	10.36±1.97
	0400	8.72±1.53	10.91±1.61***
	0800	10.14±3.41	12.43±2.69*
Rec1	1200	10.55±4.74	11.72±4.77
	1600	10.24±2.00	10.39±2.79
	2000	12.36±1.32	12.10±2.83
	2400	12.48±1.06	12.31±1.60
	0400	11.91±1.53	11.84±1.79
	0800	10.75±2.24	10.68±1.44
Rec2	1200	12.35±3.59	12.26±4.48
	1600	14.00±2.54	14.47±3.50
	2000	14.18±1.39	14.83±2.15
	2400	13.92±1.25	14.06±1.74
	0400	12.82±1.59	13.38±2.08
	0800	12.29±2.17	12.63±1.64

* significantly different @ $p<0.05$
** significantly different @ $p<0.01$
*** significantly different @ $p<0.001$

Pe - pre-exposure day
Exp - exposure day
Rec - recovery day

TABLE C 4-h MEAN RESPIRATION RATES (± σ) OF Gnorimosphaeroma oregonensis (μL O_2/g wet wt/min)

Experiment P-1: Physically Dispersed Prudhoe Bay Crude Oil: 24-h Exposure at 13.2 ppm (see Figure 8B)

Day	Time	Control	Treatment
Pe1	1200	8.08±3.26	8.79±3.82
	1600	9.36±2.99	8.49±3.45
	2000	6.95±1.29	6.38±1.13
	2400	7.28±0.51	6.89±0.36*
	0400	7.80±0.91	6.59±1.75*
	0800	6.45±2.59	7.24±2.47
Pe2	1200	5.81±1.00	5.69±1.08
	1600	7.54±2.00	6.77±2.35
	2000	5.52±1.04	6.19±1.36
	2400	6.45±0.53	6.73±0.49
	0400	6.49±1.00	6.68±1.24
	0800	6.43±1.96	6.40±1.72
Exp1	1200	7.19±4.56	8.10±2.82
	1600	6.15±2.76	7.83±3.61
	2000	8.13±3.29	8.95±3.04
	2400	6.39±1.20	7.82±1.13**
	0400	6.11±2.37	7.64±3.32
	0800	7.98±3.54	7.64±2.42
Rec1	1200	6.53±2.96	9.41±3.46
	1600	8.99±4.11	7.75±3.69
	2000	7.16±3.15	8.55±2.27
	2400	7.24±0.91	10.28±1.39***
	0400	7.69±2.39	10.49±3.15*
	0800	9.80±3.09	7.80±2.71
Rec2	1200	9.23±4.69	8.95±3.36
	1600	8.22±2.84	10.17±3.29
	2000	7.03±1.88	9.04±2.68*
	2400	7.24±0.84	10.63±1.46***
	0400	7.60±2.53	9.84±2.42*
	0800	8.30±3.04	9.79±2.82

* significantly different @ $p<0.05$
** significantly different @ $p<0.01$
*** significantly different @ $p<0.001$

TABLE D 4-h MEAN RESPIRATION RATES (± σ) OF Gnorimosphaeroma oregonensis (μL O_2/g wet wt/min)

Experiment C-4: Chemically Dispersed Prudhoe Bay Crude Oil: 24-h Exposure at 1.1 ppm (see Figure 9A)

Day	Time	Control	Treatment
Pe1	1200	8.64±3.22	9.02±3.42
	1600	8.84±1.74	8.03±1.79
	2000	11.33±2.24	11.25±1.40
	2400	10.74±0.95	10.49±1.34
	0400	9.52±1.60	9.07±2.63
	0800	7.63±1.36	8.66±2.28
Pe2	1200	8.38±1.10	8.61±1.93
	1600	8.43±1.90	8.49±1.35
	2000	10.84±2.19	11.00±2.35
	2400	10.01±2.09	9.73±1.02
	0400	9.54±1.62	9.69±1.45
	0800	7.09±1.79	7.74±1.46
Exp1	1200	7.82±2.73	7.60±2.54
	1600	8.99±2.29	9.62±1.77
	2000	10.36±2.50	10.93±2.43
	2400	9.91±1.48	10.72±1.17
	0400	9.41±1.37	9.76±2.31
	0800	8.91±2.07	9.12±2.17
Rec1	1200	10.94±3.47	11.68±4.62
	1600	8.05±1.80	9.35±2.00
		POWER FAILURE	
	1100	11.03±1.44	11.50±0.94
REC1	1200	11.29±3.34	11.34±4.00
	1600	10.79±2.33	11.12±4.16
	2000	11.38±2.88	11.98±2.27
	2400	12.08±1.42	12.13±1.41
	0400	11.76±1.46	11.76±1.16
	0800	11.12±2.74	11.38±2.17

* significantly different @ $p<0.05$
** significantly different @ $p<0.01$
*** significantly different @ $p<0.001$

TABLE E 4-h MEAN RESPIRATION RATES (± σ) OF Gnorimosphaeroma oregonensis (μL O_2/g wet wt/min)

Experiment C-2: Chemically Dispersed Prudhoe Bay Crude Oil: 24-h Exposure at 6.6 ppm (see Figure 9B)

Day	Time	Control	Treatment
Pe1	1200	8.84±3.51	9.32±4.16
	1600	9.97±1.25	10.00±1.10
	2000	8.59±0.87	8.51±0.91
	2400	8.71±0.89	8.49±0.78
	0400	7.96±1.29	7.56±1.21
	0800	6.02±1.69	6.18±1.43
Pe2	1200	6.94±1.85	6.88±1.93
	1600	8.61±0.72	8.50±0.96
	2000	7.66±1.51	7.54±1.41
	2400	7.38±1.16	7.09±0.67
	0400	7.22±2.04	6.76±1.63
	0800	6.15±1.93	6.14±1.00
Exp1	1200	6.53±0.97	7.29±0.96*
	1600	7.28±0.79	7.90±0.76*
	2000	6.11±0.73	8.08±0.76***
	2400	6.42±1.04	8.39±1.26***
	0400	6.13±1.07	8.30±1.61***
	0800	5.89±1.18	8.30±1.00***
Rec1	1200	6.15±2.96	10.33±3.24***
	1600	6.95±1.44	9.54±1.41***
	2000	7.20±0.90	10.35±1.00***
	2400	7.25±1.30	9.74±0.99***
	0400	7.12±1.23	9.20±1.33***
	0800	6.96±2.21	10.19±2.99**
Rec2	1200	7.34±2.37	10.20±1.59***
	1600	8.66±1.74	10.53±1.57**
	2000	9.08±0.99	10.79±1.56***
	2400	8.58±0.78	9.35±0.80*
	0400	7.53±1.58	9.11±1.31**
	0800	7.01±2.27	9.66±4.93**

* significantly different @ $p<0.05$
** significantly different @ $p<0.01$
*** significantly different @ $p<0.001$

TABLE F 4-h MEAN RESPIRATION RATES (± σ) OF Gnorimosphaeroma oregonensis (μL O_2/g wet wt/min)

Experiment P-2: Physically Dispersed Prudhoe Bay Crude Oil: 48-h Exposure at 2.2 ppm (see Figure 10A)

Day	Time	Control	Treatment
Pe1	1200	6.25±1.65	6.22±1.36
	1600	6.44±1.15	6.74±1.06
	2000	7.17±1.04	7.25±0.95
	2400	6.80±0.97	6.72±0.93
	0400	6.17±0.96	6.18±0.80
	0800	7.20±1.34	6.97±1.49
Pe2	1200	6.30±1.33	6.08±1.23
	1600	6.41±1.10	6.92±0.92
	2000	7.39±1.05	7.11±0.77
	2400	7.11±1.12	6.98±0.72
	0400	6.68±1.35	6.69±0.59
	0800	6.60±2.72	6.41±1.85
Exp1	1200	7.35±1.89	7.16±1.41
	1600	7.60±1.58	7.58±0.74
	2000	7.44±1.62	7.67±1.60
	2400	7.56±1.09	7.65±0.85
	0400	7.32±1.57	7.75±1.07
	0800	6.23±2.16	8.13±1.74*
Exp1	1200	7.02±1.34	7.97±1.02*
	1600	7.92±1.04	9.11±1.27**
	2000	7.79±1.49	9.26±1.38**
	2400	7.94±0.98	9.76±1.06***
	0400	7.26±1.36	9.47±1.67***
	0800	7.00±2.80	9.43±2.28*
Rec1	1200	7.56±1.97	10.16±4.15*
	1600	7.90±1.55	6.99±1.06
	2000	8.54±1.45	7.56±1.62
	2400	8.67±1.19	7.48±0.85**
	0400	8.22±1.63	7.07±0.86*
	0800	7.52±1.62	7.01±1.27

TABLE F 4-h MEAN RESPIRATION RATES (± σ) OF Gnorimosphaeroma oregonensis ($\mu L\ O_2$/g wet wt/min) (cont'd.)

Experiment P-2: Physically Dispersed Prudhoe Bay Crude Oil: 48-h Exposure at 2.2 ppm (see Figure 10A)

Day	Time	Control	Treatment
Rec 2	1200	10.18±2.08	9.57±2.55
	1600	7.80±1.18	7.33±1.00
	2000	7.81±1.14	7.41±1.37
	2400	7.79±0.72	7.24±0.55**
	0400	6.44±1.21	6.21±0.98
	0800	5.53±1.38	4.99±1.26

* significantly different @ $p<0.05$
** significantly different @ $p<0.01$
*** significantly different @ $p<0.001$

TABLE G 4-h MEAN RESPIRATION RATES (± σ) OF Gnorimosphaeroma oregonensis ($\mu L\ O_2$/g wet wt/min)

Experiment P-4: Physically Dispersed Prudhoe Bay Crude Oil: 48-h Exposure at 13.2 ppm (see Figure 10B)

Day	Time	Control	Treatment
Pe1	1300	9.12±3.63	8.18±2.49
	1600	12.11±1.65	11.94±1.65
	2000	9.79±2.58	9.53±1.76
	2400	10.16±3.45	9.18±2.90
	0400	10.06±1.60	10.39±1.41
	0800	9.61±1.73	9.57±1.00
Pe2	1200	9.49±2.16	8.76±1.83
	1600	10.58±2.25	10.27±2.22
	2000	9.48±1.21	9.88±0.90
	2400	10.13±0.97	9.86±0.84
	0400	9.88±1.51	9.67±0.53
	0800	8.57±2.30	9.14±1.14
Exp1	1200	8.65±1.92	10.08±1.10*
	1600	9.36±1.50	10.41±1.08*
	2000	9.90±1.35	11.46±0.94***
	2400	10.06±1.93	11.59±2.00*
	0400	9.82±1.59	12.04±1.61***
	0800	8.98±3.89	11.57±1.35*
Exp2	1200	10.21±0.66	12.74±0.92***
	1600	9.18±1.34	12.11±1.66***
	2000	9.89±1.50	13.61±1.50***
	2400	10.19±1.76	14.24±2.49***
	0400	9.53±1.99	14.31±2.22***
	0800	8.57±2.12	11.85±2.28***
Rec1	1200	9.03±2.11	9.61±3.47
	1600	9.30±1.52	7.10±1.88**
	2000	9.96±2.32	8.65±1.56
	2400	10.14±1.51	8.66±1.05**
	0400	9.64±2.00	8.23±1.07*
	0800	8.71±2.16	7.99±2.19

TABLE G 4-h MEAN RESPIRATION RATES (± σ) OF Gnorimosphaeroma oregonensis (μL O_2/g wet wt/min) (cont'd.)

Experiment P-4: Physically Dispersed Prudhoe Bay Crude Oil: 48-h Exposure at 13.2 ppm (see Figure 10B)

Day	Time	Control	Treatment
Rec2	1200	8.40±3.15	7.89±2.39
	1600	8.41±1.88	7.96±1.72
	1800	8.96±2.48	8.65±2.03
	2400	9.21±1.55	8.69±1.16
	0400	8.37±1.67	7.67±1.52
	0800	8.52±3.47	7.81±2.48

* significantly different @ $p<0.05$
** significantly different @ $p<0.01$
*** significantly different @ $p<0.001$

TABLE H 4-h MEAN RESPIRATION RATES (± σ) OF Gnorimosphaeroma oregonensis ($\mu L\ O_2$/g wet wt/min)

Experiment C-3: Chemically Dispersed Prudhoe Bay Crude Oil: 48-h Exposure at 1.1 ppm (see Figure 11A)

Day	Time	Control	Treatment
Pe1	1200	8.71±3.32	7.09±2.78
	1600	8.86±1.63	9.45±1.40
	2000	10.14±1.18	8.84±1.05***
	2400	7.84±1.73	7.13±1.11
	0400	5.19±2.40	5.50±1.87
	0800	6.69±2.38	6.81±2.21
Pe2	1200	6.07±2.60	6.63±1.84
	1600	8.81±1.57	9.19±1.26
	2000	9.48±1.16	9.04±1.80
	2400	7.75±1.22	7.77±1.25
	0400	7.02±1.85	7.46±1.73
	0800	9.02±2.09	8.90±2.38
Exp1	1200	7.39±2.17	7.52±1.15
	1600	7.21±1.35	8.13±1.04*
	2000	9.61±2.00	10.20±1.91
	2400	8.33±1.90	9.03±1.09
	0400	7.81±1.53	8.66±1.72
	0800	7.24±1.85	8.49±2.34
Exp2	1200	7.64±2.57	8.41±1.91
	1600	7.30±1.70	8.91±1.52**
	2000	9.13±2.64	10.21±3.05
	2400	8.79±1.27	10.10±1.02**
	0400	7.74±1.11	9.54±1.90**
	0800	7.36±1.16	9.58±1.40***
Rec1	1200	7.45±2.54	9.22±2.89
	1600	7.48±1.69	9.62±2.25**
	2000	9.42±2.45	9.96±2.97
	2400	8.53±1.41	9.71±1.14*
	0400	7.04±1.22	7.99±1.56
	0800	5.76±0.98	7.01±1.42*

TABLE H 4-h MEAN RESPIRATION RATES (± σ) OF Gnorimosphaeroma oregonensis (μL O_2/g wet wt/min) (cont'd.)

Experiment C-3: Chemically Dispersed Prudhoe Bay Crude Oil: 48-h Exposure at 1.1 ppm (see Figure 11A)

Day	Time	Control	Treatment
Rec2	1200	6.04±1.98	7.21±2.48
	1600	6.09±1.12	7.23±1.31*
	2000	8.81±2.01	8.68±1.98
	2400	6.53±1.52	6.76±2.04
	0400	6.01±1.79	6.83±2.22
	0800	5.69±2.72	6.44±3.20

* significantly different @ $p<0.05$
** significantly different @ $p<0.01$
*** significantly different @ $p<0.001$

TABLE I 4-h MEAN RESPIRATION RATES (± σ) OF Gnorimosphaeroma oregonensis (μL O_2/g wet wt/min)

Experiment C-1: Chemically Dispersed Prudhoe Bay Crude Oil: 48-h Exposure at 6.6 ppm (see Figure 11B)

Day	Time	Control	Treatment
Pe1	1200	7.42±3.50	7.80±4.77
	1600	9.03±1.25	9.52±1.57
	2400	8.54±0.89	7.81±1.10
	0400	7.93±0.90	7.43±1.04
	0800	7.64±0.99	7.61±1.60
Pe2	1200	7.43±1.72	7.41±0.84
	1600	8.39±0.61	8.64±0.47
	2000	9.36±0.95	9.69±0.67
	2400	8.49±1.33	8.66±0.91
	0400	7.96±1.12	8.08±0.66
	0800	6.74±1.26	6.73±1.63
Exp1	1200	7.58±1.94	7.74±1.68
	1600	7.88±0.87	8.51±1.34
	2000	8.19±0.71	10.69±0.83***
	2400	7.48±1.26	10.82±1.20***
	0400	7.11±2.15	10.22±1.60***
	0800	7.68±1.33	11.67±2.37***
Exp2	1200	8.22±1.90	11.37±4.22*
	1600	7.46±1.19	10.94±1.88***
	2000	8.29±1.20	12.54±1.18***
	2400	8.47±1.51	13.09±1.44***
	0400	8.20±1.88	14.06±2.12***
	0800	7.11±1.62	12.97±2.49***
Rec1	1200	6.30±1.30	7.93±2.19*
	1600	6.43±1.64	7.23±1.15
	2000	7.66±2.49	8.60±3.02
	2400	7.50±1.04	7.86±1.79
	0400	7.22±1.70	7.88±1.78
	0800	8.89±2.84	10.04±1.96

TABLE I 4-h MEAN RESPIRATION RATES (± σ) OF Gnorimosphaeroma oregonensis (μL O_2/g wet wt/min) (Cont'd.)

Experiment C-1: Chemically Dispersed Prudhoe Bay Crude Oil: 48-h Exposure at 6.6 ppm (see Figure 11B)

Day	Time	Control	Treatment
Rec2	1200	8.54±1.44	9.37±1.52
	1600	7.73±1.36	9.41±2.11*
	2000	8.30±1.13	8.84±1.95
	2400	7.99±0.82	8.91±2.03
	0400	7.78±1.56	9.38±1.56*
	0800	7.09±2.00	8.45±2.92

* significantly different @ $p<0.05$
** significantly different @ $p<0.01$
*** significantly different @ $p<0.001$

TABLE J RESIDUAL NAPHTHALENES (ppm) IN TISSUES* OF THE ISOPOD Gnorimosphaeroma oregonensis FOLLOWING 24-h EXPOSURES TO PHYSICALLY AND CHEMICALLY DISPERSED OIL

Weeks After Exposure	Control	Physically Dispersed Oil (18 ppm)	Chemically Dispersed Oil (9 ppm)
1	0.40	0.60	0.90
2	0.40	0.60	0.60
3	0.40	1.10	0.70
4	2.30	0.60	1.90
5	1.00	1.00	1.00

* sample wet weights = 0.5 - 1.2 g

TABLE K MEAN LENGTH, DRY WEIGHT AND MOULTING FREQUENCY OF THE ISOPOD Gnorimosphaeroma oregonensis FOLLOWING 24-h EXPOSURES TO PHYSICALLY AND CHEMICALLY DISPERSED OIL

i. MEAN LENGTH ± σ (mm)
(n=10)

Weeks After Exposure	Control		Physically Dispersed Oil (18 ppm)		Chemically Dispersed Oil (9 ppm)	
	Females	Males	Females	Males	Females	Males
1	4.8±0.4	8.1±0.8	5.0±0.8	8.2±0.9	5.1±0.5	8.2±0.4
2	5.1±0.5	8.1±0.7	5.3±0.4	7.6±0.7	5.0±0.5	7.9±1.2
3	5.2±0.4	7.5±1.2	5.3±0.6	7.6±0.5	4.7±0.6	8.0±0.8
4	4.8±0.4	7.5±0.6	5.0±0.5	7.7±0.7	4.7±0.6	8.0±0.8
5	5.0±0.6	7.7±0.3	5.3±0.4	7.9±0.5	5.5±0.7	7.9±0.5
6*	5.3±0.8	-	5.1±0.4	-	5.2±0.6	-
7*	5.1±0.4	-	5.1±0.4	-	5.2±0.5	-

*n=20 (complete male mortality)

TABLE K MEAN LENGTH, DRY WEIGHT AND MOULTING FREQUENCY OF THE ISOPOD Gnorimosphaeroma oregonensis FOLLOWING 24-h EXPOSURES TO PHYSICALLY AND CHEMICALLY DISPERSED OIL (Cont'd.)

ii. MEAN DRY WEIGHT (mg)

Weeks After Exposure	Control	Physically Dispersed Oil (18 ppm)	Chemically Dispersed Oil (9 ppm)
1	7.41	7.50	6.60
2	7.60	7.53	6.56
3	6.74	7.01	7.86
4	6.70	6.04	6.44
5	6.53	6.86	6.82

iii. MOULTING FREQUENCY (no. of moults per tank)

Weeks After Exposure	Control	Physically Dispersed Oil (18 ppm)	Chemically Dispersed Oil (9 ppm)
1	+100	+100	+100
2	27	20	25
3	10	20	5
4	10	16	10
5	2	-4	9

TABLE L JUVENILES PER FEMALE ISOPOD (Gnorimosphaeroma oregonensis) PRESENT IN WEEK FOUR FOLLOWING 24-h EXPOSURES TO PHYSICALLY AND CHEMICALLY DISPERSED OIL

Weeks After Exposure	Control	Physically Dispersed Oil (18 ppm)	Chemically Dispersed Oil (9 ppm)
4	0.11	0.02	0.04
5	0.80	1.44	0.40
6	2.31	3.63	3.60
7	3.28	5.14	4.49
8	3.14	4.95	4.96
9	4.45	5.09	4.03

TABLE M PERCENT MORTALITY OF THE ISOPOD Gnorimosphaeroma oregonensis FOLLOWING 24-h EXPOSURES TO PHYSICALLY AND CHEMICALLY DISPERSED OIL

Percent Mortality of Remaining Population

Weeks After Exposure	Control		Physically Dispersed Oil (18 ppm)		Chemically Dispersed Oil (9 ppm)	
	Females	Males	Females	Males	Females	Males
1	3.4	18.0	4.9	20.4	3.5	14.5
2	2.3	8.7	2.2	10.5	2.1	12.7
3	6.0	32.9	2.7	17.0	4.1	25.1
4	5.3	29.8	2.7	43.5	3.9	34.7
5	4.6	35.9	1.2	25.0	2.8	35.7
6	0	40.0	2.2	25.0	3.9	71.4